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NAME OF AUTHOR/*NOM DE L'AUTEUR* R.I. Kennedy

TITLE OF THESIS/*TITRE DE LA THÈSE* KINETICS, DISTRIBUTION AND EXCRETION OF TRIIODOTHYRONINE IN SHEEP

UNIVERSITY/*UNIVERSITÉ* University of Alberta

DEGREE FOR WHICH THESIS WAS PRESENTED/*GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE* Master of Science

YEAR THIS DEGREE CONFERRED/*ANNÉE D'OBTENTION DE CE GRADE* 1979

NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* B.A. Young

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KINETICS, DISTRIBUTION AND EXCRETION OF TRIIODOTHYRONINE IN SHEEP

by

R.I.KENNEDY

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

ANIMAL PHYSIOLOGY

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL 1979

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled KINETICS, DISTRIBUTION AND EXCRETION OF TRIIODOTHYRONINE IN SHEEP submitted by R.I.KENNEDY in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL PHYSIOLOGY.

.....
Supervisor

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Date July 26, 1979

ABSTRACT

Experiments were conducted on sheep to determine; (i), if level of feed intake altered their thyroid status; (ii), if it was possible for triiodothyronine (T3) to enter the rumen of sheep; (iii), what metabolites of T3 were excreted in urine and feces; and (iv), if the rumen microbiota could metabolize T3.

Yearling, rumen-fistulated, Suffolk-cross ewes were fed either a maintenance or twice maintenance ration. After at least three weeks of adaptation, catheters were inserted into each jugular vein, serum samples for T3, thyroxine (T4), and iodine analyses were collected. Iodine-125 labelled T3 (1,000,000,000 cpm) was injected through one jugular catheter and serial blood samples (for the preparation of serum) were collected from the other. Serial samples of saliva, rumen fluid, urine and feces were also collected. Total radioactivity in each serum sample was determined, and components were separated by thin layer chromatography. Serum T3 and T4 concentrations were determined by radioimmunoassay. Serum iodine was determined by chemical means. In addition, an <u>in-vitro</u> experiment was performed to determine if T3 was metabolized by rumen the microbiota.

The two levels of feed intake did not cause significant changes in T3 or T4 concentration or T3 disappearance rate. Iodine concentration was significantly lower in the sheep receiving the high level of feed intake. The calculated

distribution volume (i.e. the kinetic pool) of T3 in the sheep was significantly greater in the twice maintenance feed level group. T3 was not detectable in either saliva or rumen fluid. A fraction of the total I-125 that appeared in the urine was not chemically identified. This fraction appeared to be a major metabolite (or metabolites) of T3. The in-vitro experiment showed that unautoclaved rumen fluid bound or degraded T3 to a greater extent than did autoclaved rumen fluid.

It was concluded that the level of feed intake (either maintenance or twice maintenance) did not cause significant changes in common indices of thyroid status but that there was a significant effect on the T3 distribution volume. This is indicative of changes in tissue binding of T3. T3 was not detectable in the rumen. However, the possibility that it was associated with microbes attached to the rumen epithelium was not eliminated.

ACKNOWLEDGEMENTS

I would like thank Dr. R.T. Berg and Dr. L.P. Milligan, Chairman and former Chairman of the Department of Animal Science for placing the facilities of the Department at my disposal.

I wish to thank Dr. B.A. Young, Professor of Animal Physiology, for his advice and suggestions during the course of this work.

I wish to thank Mr. R. Weingardt and Dr. R.T. Hardin for their assistance with the statistical analyses and computation.

I wish to acknowledge the assistance of Mr. B.V. Turner with the handling of the isotopes used. I also wish to thank Mr. J. Francis, Mr. L. Gedge and Mrs. J. Morgan Jones for their care and feeding of the animals.

I would like to thank my fellow students for their assistance during the collection of samples and preparation of the text.

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0.1 CHAPTER 1 : INTRODUCTION AND REVIEW OF LITERATURE

0.1.1 INTRODUCTION

The interrelationships between thyroid hormones and digestive function in animals are complex. For example, during exposure of sheep to cold ambient temperatures circulating levels of thyroid hormones increase, rate of passage of digesta through the alimentary tract increases and apparent digestibility of food decreases (Christopherson, 1976; Westra and Christopherson, 1976; Kennedy et al, 1977). The implication is that increased thyroid function is responsible for the increased rate of passage and the concommitant decreases in digestibility was supported by Kennedy et al, (1977) who administered triiodothyronine (T3) to thyroidectomized sheep and produced increased rate of passage and decreased digestibility of the food. The effect of high ambient temperature on thyroid function of sheep appears at least superficially, to be the opposite to the effect of low ambient temperature. Sanchez and Evans (1972) reported that when sheep were exposed to above thermoneutral temperatures, there was a decrease in serum thyroxine (T4) levels and a decreased T4 secretion rate. Furthermore, when sheep in a thermoneutral environment were fed the same amount of feed that the sheep at the high temperature consumed ad lib, their thyroid status changed in a manner that was analagous to the changes observed in the heat stressed animals. There is evidence that acute feed

restriction causes a decrease in T4 secretion rate in goats (Abdullah and Falconer, 1977) and that long term energy restriction in pigs results in a decreased rate of I-125 labelled T4 disappearance from their plasma (Ingram and Kaciuba-Uscilko, 1977).

One area of thyroid hormone metabolism and digestive function that has not been investigated is the possibility of thyroid hormones entering the rumen and acting directly on the rumen microbiota. O'Connor et al (1970) suggested that steroid hormones or steroid hormone analogs could change the metabolic products of the rumen microbiota, also Biswas (1975) showed that T4 could be used to stimulate growth of Escherichia coli.

Studies were undertaken to determine if level of food intake caused measurable effects on T3 or T4 serum concentration, T3 disappearance rate or distribution volume. At the same time an attempt was made to detect T3 in rumen fluid, urine and feces.

When this experiment was initially designed, the primary purpose was to determine if T3 entered the rumen of sheep. The reason for this was based on the hypothesis that T3 may be capable of influencing the rumen microbiota and consequently influence digestive function in ruminants. It was also decided to determine if two levels of feed intake could alter thyroid status and therefore these treatments were imposed. At the same time it was decided to gather some information on the form and route of excretion of T3 from

sheep. Because of these three objectives this thesis has been written in the form of an Introduction and Review of Literature followed by chapters on each phase of the experiment and a General Conclusion and Discussion.

0.1.2 LITERATURE REVIEW

Iodine and thyroid hormone metabolism are closely related. In fact, the synthesis of thyroid hormones in the thyroid gland is generally believed to be the only route of iodine uptake and incorporation into animal tissue. A possible exception is the microbiocidal effect in leucocytes (Pincus and Klebanoff, 1971). This literature review will discuss iodine absorption, distribution and metabolism with respect to the thyroid hormones and their physiological function in domestic animals with emphasis on sheep. Literature on lab animals and humans is also discussed where relevant information is not available for domestic animals.

0.1.2.1 ABSORPTION AND DISTRIBUTION OF IODINE

Iodine is secreted into the alimentary tract by the salivary glands and abomasum. It is absorbed by the small intestines and is found in all tissues of the body. In most tissues the concentration of iodine is lower than the circulating level. The exceptions are thyroid tissue, salivary glands, placental membranes, lactating mammary glands and inflamed tissues (Miller *et al*, 1975; McGuire and Burman, 1978). The above information was determined by

isotope tracer techniques and therefore may not represent the "true" iodine pool as iodine may require a long time period to equilibrate (Cottino et al, 1972). In mouse muscle following long term (3 month) constant feeding of I-125, equilibrium was not achieved. This was shown by the differences in estimates of muscle iodine concentration based on isotope and chemical determinations (by neutron activation analyses) which showed a 4 to 6 fold discrepancy between stable iodine and estimates based on radioactivity. This could be explained by a slow turnover pool in muscle (Cottino et al, 1972) and shows that labelled iodine is slow to equilibrate with the total iodine pool. Consequently, studies that do not allow sufficient time for labelled iodine to become uniformly mixed with the total iodine pool may produce erroneous data.

Iodine can be conserved by recycling it through the alimentary tract creating a large gastrointestinal iodine pool. By recycling iodine through the alimentary tract, animals can decrease urinary iodine loss especially under conditions of low iodine intake (Miller et al, 1975). In the abomasum, the flow of iodine across the abomasal wall into the lumen can be eighteen times the flow in the opposite direction. Iodine is then reabsorbed from the intestines (Miller et al, 1975).

0.1.2.2 EXCRETION OF IODINE

The rate of urinary excretion of inorganic iodine

parallels the plasma non-protein bound iodine concentration and is increased by high dietary chloride and iodide (Miller et al 1975). Thyroid hormones and their sulfonic and glucuronic acid conjugates are excreted in urine (Shakespear and Burke, 1976; Burke and Shakespear, 1976). T3 is excreted at a rate that parallels the excretion of creatinine, which implies that it is not actively reabsorbed by the kidneys. However, it has been reported that T4 is actively reabsorbed (Rogowski et al 1978).

Both hormonal and inorganic iodine is excreted in feces, most of which is derived from thyroid hormones which enter the small intestines as free hormones or as sulfonic or glucuronic acid conjugates from the liver (Miller et al, 1975; Ramsden, 1977). It would then be possible for the conjugated hormones to be deconjugated in the gut and be reabsorbed. However, as Ramsden (1977) points out, the existence of this "entero-hepatic" cycle has not been confirmed.

0.1.2.3 SYNTHESIS AND METABOLISM OF THYROID HORMONES

Iodide in the thyroid gland is oxidized and bound to tyrosine molecules which are attached to thyroglobulin. The resulting products are mono- and diiodotyrosines. Diiodotyrosine (or monoiodotyrosine and a diiodotyrosine) molecules are then joined together producing a molecule of T4 (or T3) which is attached to a thyroglobulin molecule. These molecules are released when the thyroglobulin is

hydrolised by hydrolytic enzymes. Ekholm (1977) studied the process in detail.

Following release from the thyroid gland, T4 and T3 are bound to proteins in the blood. Plasma proteins have a greater affinity for T4 than T3. The binding of these hormones to large proteins limits urinary loss as well as acting as a buffer that maintains a relatively constant free hormone level (Bernal and Refetoff, 1977). Binding proteins are also found within cells where the T3 is bound more strongly than T4. Consequently, 90% of the extrathyroidal T3 is intracellular while only 60% of the extrathyroidal T4 is within cells. Intracellular binding of thyroid hormones was reviewed by Bernal and Refetoff (1977).

T4 and its derivatives are deiodinated, decarboxylated, deaminated and (or) conjugated in most tissues of the body. The resulting products either have specific functions or are rapidly excreted (Ramsden 1977). Some of these products are T3, 3'5'3-triiodothyronine (reverse T3 or rT3), 3',3-diiodcthyronine (3',3-T2), 3',5',3,5-tetraiodothyroacetic acid (TETRAC), 3',3,5-triiodothyroacetic acid (TRIAC) and conjugation products of T3 and T4. T3 is generally considered to be the most active of the thyroid hormones. It causes an increased metabolic rate and is also associated with increased nucleic acid and protein synthesis (Oppenheimer et al 1975). Reverse T3 has been associated with a feedback mechanism that controls the enzymatic production of T3 from T4, this has

been reviewed by Ramsden (1977). Metabolism of T3, T4 and rT3 in fetal and adult sheep was studied by Chopra et al (1975). In fetal sheep serum concentrations of T4 and rT3 were greater than in adult sheep, serum T3 was lower in the fetal sheep. The hormone concentrations in the fetal sheep changed to be similiar to the adult levels shortly after birth, this indicates that the metabolism of of T3, T4 and rT3 are not independent. TETRAC which is associated with regulation of cyclic nucleotide diphosphoesterase, has been to lower blood cholesterol (Ramsden 1977). There has been no report on the role of TRIAC in the regulation of metabolism. Likewise, no reports have been made on the role of 3',3-T2, a deiodination product of both T3 and rT3 (Chopra et al 1978; Nakamura et al, 1978). The mechanisms by which thyroid hormones control metabolism are reviewed and discussed by Bernal and Refetoff (1977) and Oppenhiemer et al (1975). The sulfonic and glucuronic acid conjugates of thyroid hormones are produced and excreted by both the kidneys and liver. They are nct normally found in the blood and probably are nonabsorbable (by tissue cr prctein) excretion products of thyroid hormones (Ramsden 1977; Shakespear and Burke 1976).

In general, sequential monodeiodination is a major path of thyroid hormone metabolism (Rudolf et al, 1976). The processes are probably not random but are controlled and catalysed by specific enzymes that produce specific products (Ramsden, 1977; Soffer et al, 1973).

0.1.2.4 KINETICS OF THYROID HORMONES IN DOMESTIC ANIMALS

The kinetics of thyroid hormones, particularly disappearance rate, have been investigated by a number of researchers. Some of their results are shown in Table 1. Commonly used parameters utilized in studies of thyroid hormone kinetics are metabolic clearance rate (MCR), a measurement of the rate at which blood is cleared of the hormone; half-life (T1/2), the time required for one half of the hormone to be removed from circulation; turnover (t), the length of time an average molecule of the hormone stays in the pool; and disappearance rate constant (k), the fraction of total hormone that disappears per unit of time. The relationship of these parameters are $T1/2=(.693)/(k)$ and $t=(1.44)T1/2$. MCR is not readily converted to the other parameters because the hormone outside the circulatory system is not directly involved.

All values in Table 1 were obtained by using isotope tracer techniques. These methods require that the radioactivity be isolated or separated so that only the compound of interest is counted. As mentioned previously, deiodination is the major path of thyroid hormone metabolism. Therefore, when iodine labelled thyroid hormones are used in kinetic studies, iodide must be removed prior to counting of activity. Since deiodination occurs one step at a time, metabolites would be present, which also should be removed.

In previous studies some methods used to determine the

Table 1 Estimates of disappearance rate of triiodothyronine (T3) and thyroxine (T4) in sheep and pigs

	Ambient environment (C)	Feeding level	Disappearance rate (/h) T3	T4	Reference
Pregnant sheep	14-29	?	0.099		Dussault et al (1975)
Fetal sheep			0.126		Dussault et al (1975)
Mature rams	20	ad lib		0.019	Sanchez and Evans (1972)
Mature rams	32[1]	ad lib		0.017	Sanchez and Evans (1972)
Young pig	32	3% BW[2]		0.029	Evans and Ingram (1977)
Young pig	32	6% BW		0.034	Evans and Ingram (1977)
Young pig	8	3% BW		0.028	Evans and Ingram (1977)
Young pig	8	6% BW		0.056	Evans and Ingram (1977)
Young pig	25	3% BW		0.038	Ingram and Kaciuba-Uscilko (1977)
Young pig	25	6% BW		0.043	Ingram and Kaciuba-Uscilko (1977)
Young pig	25	3% BW+3%[3] straw		0.029	Ingram and Kaciuba-Uskilko (1977)

1) Rams acclimated to 20C were acutely exposed to 32C

2) The pigs were fed a concentrate ration at 3% or 6% of their body weight (BW)

3) The pigs were fed a concentrate ration at 3% BW plus ground straw at 3% BW

radioactivity of the labelled hormone were total radioactivity of radioactive iodine in unextracted serum or plasma, extraction of the labelled hormone with butanol or precipitation with trichloroacetic acid. Evans and Ingram (1977) and Ingram and Kaciuba-Uscilko (1977) used the total radioactivity of radioactive iodine in unextracted plasma method. Dussault et al,(1975) and Chopra et al (1975) extracted labelled hormones with butanol, Yousef and Johnson (1968) and Sanchez and Evans (1972) used trichloroacetic acid precipitated protein. None of these methods are completely satisfactory, least of all total radioactivity of radioactive iodine in unextracted serum or plasma. As butanol extraction removes iodide, this method would give more reliable results than total radioactivity. However, the deiodinated products would still be present. TCA precipitation removes iodine bound to proteins. This too would contain the metabolites of the thyroid hormones. In addition, the denaturation of the proteins may cause a change in affinity for the thyronines. Theoretically the ideal method would be one that specifically isolates the hormones so that each one could be counted individually. Chromatography is one method that has the potential to do this.

The literature values included in Table 1 are intended to show that several factors can influence disappearance rate of thyroid hormones. These include physiological state of the animal, (i.e. fetal or mature), thermal factors,

Table 2 Estimate of serum concentrations of thyroxine (T4) and triiodothyronine (T3) in sheep exposed to various ambient temperatures and offered various rations

Ambient temperature (C)	Ration	T4 ug/100ml	T3 ng/100ml	Reference
0.8	Pelleted ration	12.8	242	Westra and Christopherson (1976)
10.0	Pelleted ration	11.8	192	Westra and Christopherson (1976)
17.7	Pelleted ration	8.7	123	Westra and Christopherson (1976)
0.8	Hay ration	10.6	120	Westra and Christopherson (1976)
10.0	Hay ration	10.1	161	Westra and Christopherson (1976
17.7	Hay ration	7.3	73	Westra and Christopherson (1976)
22-25	Pelleted hay	8.0	62	Kennedy et al (1977)
2-5	Pelleted hay	13.5	152	Kennedy et al (1977)
20	ad lib	7.9		Sanchez and Evans (1972)
32[1]	ad lib	7.6		Sanchez and Evans (1972)
20[1]	restricted	6.4		Sanchez and Evans (1972)

1) The sheep at 20C were pair fed with those at 32C

energy intake, species and hormone in question i.e. T3, T4 etc. Another factor that can influence the apparent disappearance rate is the method used for their determination (as indicated above).

Some of the effects of different types and amounts of rations and ambient temperatures are illustrated in Table 2. thyroid hormones in sheep on various experimental treatments. Data in this table illustrate that ambient temperature, food intake and type of food cause changes in the circulating concentrations of both T3 and T4. The concentration of these hormones increases as ambient temperature decreases. T4 concentrations decrease when food intake is limited. The effect of limited food intake on T3 concentrations in sheep has not been reported. Westra and Chistopherson (1976) indicated that sheep recieving a pelleted hay ration have higher circulating concentrations of both T3 and T4 than do sheep receiving a hay ration.

In humans, symptoms of hyperthyroidism include increased appetite, weight loss and diarrhea. Hypothyroidism results in the opposite symptoms, that is there is often decreased appetite, weight gain and constipation (Merck Mannual 1972). These symptoms may be brought about by the influence of thyroid hormones on basal metabolic rate and gastrointestinal motility. More complete discussions of these topics can be found in the reviews by Ramsden (1977), Harland and Orr (1975) and Levin (1969).

The effects of fasting and starvation on thyroid status

in humans has been reviewed by Ramsden (1977) and discussed by Azizi (1978), Lee et al (1977) and Visser et al (1978). Generally, a depression of circulating levels of T3 results from starvation, fasting during dieting and psychological conditions such as anorexia nervosa (which causes severe loss of appetite).

0.1.2.5 EFFECT OF HORMONES ON THE RUMEN MICROBIOTA

The possibility that mammalian hormones may affect the metabolism of the rumen microbiota has not been extensively investigated. In 1970 O'Connor et al reported that the steroids diethylstilbestrol, desoxycortisone, hydrocortisone methandrotenalone, prednisolone and testosterone had very little effect on the products of rumen microbial metabolism. However they reported that melangesterol acetate increased volatile fatty acid production. The significance of this is unclear as no statistical data were reported. The only evidence that thyroid hormones may affect microbial metabolism was reported by Biswas (1975) who found that 0.001% T4 acted as a growth stimulant for Escherichia coli. It is unlikely that under in vivo conditions a concentration of 0.001% T4 would ever occur.

It is known that thyroid hormones can enter the hind gut of sheep (Irvine 1974), but it is not known if they can enter the rumen. Miller et al, (1975) reported that 5% of a labelled dose of T4 could be detected in the abomasum of cows.

0.2 CHAPTER 2 : EFFECT OF FEED LEVEL ON TRIIODOTHYRONINE AND THYROXINE SERUM CONCENTRATION AND TRIIODOTHYRONINE DISAPPEARANCE RATE AND DISTRIBUTION VOLUME IN SHEEP

0.2.1 ABSTRACT

One year old rumen-fistulated ewes were fed rations that corresponded to near maintenance or twice maintenance. Potassium iodide was administered intraruminally to limit iodine recycling through the thyroid. After adaption to experimental conditions, indwelling catheters were placed in each jugular vein. Serum samples for triiodothyronine (T3), thyroxine (T4), and iodine determination were collected. A tracer dose of I-125 labelled T3 was injected into one catheter and serial blood samples (for serum preparation) were taken from the other. Total radioactivity of the serum samples was determined, and radioactivity in the T3 portion was determined after separation of the radioactive fractions by thin layer chromatography of ethanol extracts of the serum. Serum T3 and T4 concentrations were determined by radioimmunoassay and iodine concentration was determined by a chemical method. Dry matter, energy and protein digestibility were also determined.

Serum T3 and T4 concentrations in the animals on the maintenance and twice maintenance levels of feed intake were not significantly different. Serum iodine concentrations were 24.6 and 15.9 ug/100ml ($P<0.05$). Digestibilities of energy were 50.3% and 41.7% and digestibilities for protein

were 71.6% and 64.3%, ($P<0.01$). Dry matter digestibilities were not significantly different. Disappearance rate of T3 in animals on each treatment was not significantly different. Distribution volume of T3 was significantly greater ($P<0.05$) in the sheep fed at the higher level.

0.2.2 INTRODUCTION

Thyroid hormones are known to alter several parameters of digestive function. These include gut fill, rate of passage, voluntary feed intake and digestibility (Miller et al, 1974; Kennedy et al, 1977). The effect of level of feed intake on thyroid status is not as well known. It has been shown that starvation and fasting cause reductions in circulating T3 levels in humans (Azizi, 1978; Lee et al, 1978; Visser et al, 1978). Sanchez and Evans (1972) found that when sheep had their ration sharply reduced there was a transitory decrease in T4 secretion rate. Similiar results were also found in rats (Yousef and Johnson, 1968), and goats (Abdullah and Falconer, 1977).

The purpose of the experiment was to determine if T3 disappearance rate, distribution volume or serum concentration could be influenced by two levels of feed intake that are considered to be normal and of common usage. Other parameters that were measured are serum iodine and T4 concentration, and digestibility of dry matter, protein and energy.

0.2.3 METHODS AND MATERIALS

Six yearling Suffolk cross ewes (27-38 kg) with permanent rumen fistulae were allotted to two treatment groups in three separate time periods (Periods 1,2 and 3). The same four sheep were used in Periods 1 and 2, in each of these periods there were two sheep in each treatment group. Two additional sheep were used in Period 3, one in each treatment group. The feed intake in the two treatments was calculated to be near maintenance and twice maintenace (760 and 1520 g alfalfa pellets per day, National Academy of Sciences, 1975). The food was dispensed in equal hourly portions by an automatic feeding system. Water was available at all times. After an initial loading dose of 15 mg KI administered intraruminally on two consecutive days, the sheep were given daily doses of 1.5 mg KI to limit iodine recycling through the thyroid gland. In addition, daily doses of 2.0 g NaCl were given. Lighting was continuous and temperature was maintained at 22-25 C. At least three weeks of adaptation were allowed to elapse before the sample collections were commenced.

After the adaption period, the sheep were prepared for the actual experiment. On the day before the start of collection of samples, one indwelling catheter was placed in each jugular vein of the sheep. Serum samples for T3, T4 and iodine determination were collected for one week. Feces were also collected for determination of digestibility. Bladder catheters were then placed in the sheep that were to receive

I-125 labelled T3 for determination of T3 disappearance rate and T3 distribution volume. The bladder catheters were removed 72 hours after the injection of the label. In order to prevent bladder and kidney infections the sheep were given intramuscular injections 3 ml/d of a combination of penicillin and streptomycin (Combiotic, Pfizer).

Iodine-125 labelled T3 (specific activity 878 uCi/ug for Period 1, 1091 uCi/ug for Periods 2 and 3) obtained from New England Nuclear, was diluted with an approximately equal volume of autogenous serum; heparinized saline was used to dilute the mixture to a total volume of approximately 10 ml. The exact dose of radioactivity injected into each animal was determined from the weight of the injectate and from the radioactivity of a 1/1000 dilution of the injectate. Purity of the injectate was determined by chromatography and found to be 90% T3 with less than 5% iodide in all cases. After injection of the labelled hormone, serial blood samples were collected from the contralateral catheter for the preparation of serum, which was then stored (for less than two months) at -20 C until analysis was done.

Dry matter of feed was determined by drying feed samples at 110 C for 3 h. Dry matter of feces was determined by drying samples in a forced air oven at 65 C for 48 h. Energy content of dried samples was determined using a Parr oxygen bomb calorimeter. Nitrogen was determined by the Kjeldahl method (Association of Official Agricultural Chemists, 1975).

Serum concentrations of T3 and T4 were determined by radioimmunoassay (Chopra et al, 1972; Calbiochem 1975); unbound label was removed with dextran coated charcoal. Serum iodine was determined by the Hycel Cuvette PBI technique (Reduction of CeIV to CeIII by AsIII, Hycel Inc 1974).

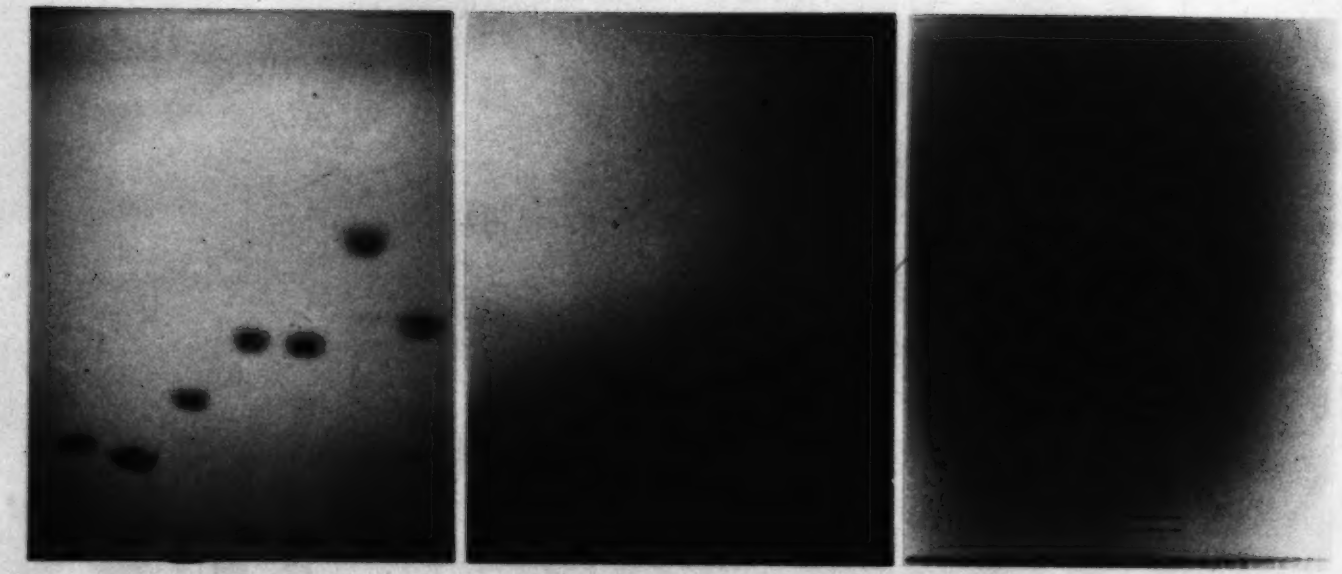
Total radioactivity in all samples was determined with an automated gamma counter (Beckman Biogamma) set for maximum efficiency. Labelled components in serum were extracted with ethanol by a modification of the method of Patel and Burger, (1973). The procedure was as follows: 0.0003 g of each of T3, T4 and KI in 0.10 ml 0.04N NaCH were added to 0.50 ml of serum. The samples were mixed with a vortex mixer for 15 seconds. After the mixing they were cooled on ice for 15 minutes, 1.20 ml 95% ethanol was added, samples were mixed with the vortex mixer for 60 seconds and centrifuged at 1190xg at room temperature for 10 minutes. The supernatant was removed and 0.10 ml of it was used for the chromatographic separation of I-125 containing compounds. This method of extraction removed 92% of tracer doses of T3 added to serum. There were no indications of preferential extraction of either T3 or iodide.

The thin layer chromatography (TLC) plates (silica gel G, manufactured by Macherey-Nagel of Duren, Germany) and solvents (t-amyl alcohol;methanol;hexane;4 N ammonium hydroxide, 6;2;1;1) corresponded to those used by Latham et al, (1976). The organic solvents were redistilled in a glass

still and the ammonium hydroxide was prepared with distilled water. The solvent was changed prior to each run. T3 and T4 were located by ultraviolet light (254 nm) quenching. Iodide was located by spraying the chromatograph with 0.1% palladium chloride in dilute HCl which caused the iodide to appear as a dark area. Separation of T3, T4 and some other thyroid hormones are shown in Figure 1. Each chromatograph was divided into ten bands, each band was removed from the TLC plate and its radioactivity determined. Tests were conducted to determine if the silica gel from the TLC plates or residues from the chromatography reduced counting efficiency. The results showed that there was no loss of efficiency.

The fraction of the total I-125 radioactivity (on the plate) in each band was determined. T3 specific activity was determined by multiplying the fraction of the radioactivity in each sample that migrated as T3 by the total I-125 radioactivity per ml of serum in the corresponding sample. When the counts in the T3 bands fell below the number required for counting a accuracy of 7% (this limit was equivalent ot approximately 2000 cpm of I-125 T3 in one ml of serum) in any sample in a period, no further values from that animal or the others in that period were used for calculation of disappearance rate and distribution volume. In Period 1 this occurred in the 24 h samples, in Period 2 in the 17 h samples and in Period 3 in the 24 h samples.

The disappearance rate and distribution volume of T3



i ii iii iv v vi vii

A B C

Figure 1 Thin layer chromatographic separation of thyroid hormones and some of their derivatives.

A) Separation of: i) diiodotyrosine, ii) monoiodotyrosine, iii) thyroxine, iv) 3,5-diiodothyronine, v) 3',3,5-triiodothyronine, vi) 3',3,5-triiodothyroacetic acid and vii) 3',5',3,5-tetraiodothyroacetic acid. Illumination is by short wave ultraviolet light (primarily 254 nm).

B) Separation of 3',3,5-triiodothyronine and thyroxine in a serum extract. Illumination is by ultraviolet light.

C) Separation of iodide (A) from 3',3,5-triiodothyronine (B) and thyroxine (C) in a serum extract. Illumination is by incandescent light.

for each sheep was determined by linear regression of the natural log of the calculated T3 specific activity against time. Distribution volume was estimated by extrapolating to determine the time zero specific activity of serum and calculating the volume of serum that would be required to contain the injected dose of radioactivity. Values used for the calculation of disappearance rate and distribution volume were; Period 1, 2.25-24 h (6 values, some samples were lost when tubes in a centrifuge broke); Period 2, (2-24 h, 6 values); Period 3, 2-24 h, (7 values).

The values chosen for the estimation of disappearance rate constant and distribution volume were based on the data from the second and third periods. The reason for this was that some of the samples from the first period were lost. Two criteria were used to determine acceptibility of values, the first was that there must be sufficient radioactivity present for counting accuracy, this criterion eliminated all values beyond the 24 h samples. Secondly, the samples collected between time zero and 2 h were not used because this period was considered to represent the mixing phase and therefore does not represent disappearance of the hormone from the sheep. The decision to use values between 2 and 24 h was initially made on the basis of graphical analysis, it was later verified statistically. The method used for the verification was to calculate the probability (for each animal) of a difference between the observed and predicted 2 h (based on regression analysis). These probabilities were

then combined by the methods described in Sokal and Rohlf (1969). It was found that the combined probabilities were not statistically significant. Similiar calculations on the 1 h samples showed that the observed radioactivity, for all animals, was greater than the predicted by at least one standard deviation. This indicates that at 1 h the labelled hormone was not completely distributed through the tissues of the sheep. Fractional disappearance rates for total I-125 radioactivity in unextracted serum over the same time period as was used for T3 and over the entire collection period were also calculated (the actual time over which this was calculated was not the same in each period, see Appendix 1).

In Period 1, T3, T4 and I concentrations in serum and digestibility of protein, dry matter and energy were determined in two animals on each treatment. T3 disappearance rate and distribution volume was determined in one animal on each treatment. In Period 2 all of the above parameters were determined in two animals in each treatment. In Period 3, there was only one animal on each treatment. Also in Period 3, serum samples were not collected for 1 week prior to the day of injection of I-125 T3, therefore serum T3, T4 and I concentrations were not used in the statistical analyses. See Tables 3 and 4 for identification and treatment of individual animals during the three periods.

0.2.4 RESULTS

In this experiment potassium iodide was administered to the animals to inhibit recycling of I-125 through the thyroid gland. Irvine (1975) considered this method to be acceptable since it does not alter the kinetics of thyroid hormones. No I-125 was detected with T4 during chromatography of serum samples, therefore it was assumed that there was no recycling of iodine from T3. Since the experiment was carried out in three periods the sheep in each period were administered KI prior to the start of each period for varying lengths of time (from 3 weeks to 17 weeks). It is believed that this may have some importance during the interpretation of the results. Samples were processed within two months of collection with most being processed in a month.

Dry matter digestibility, serum T3 and T4 concentrations were not significantly different in animals on the two treatments (Table 3), nor was serum total I-125 radioactivity or T3 associated label disappearance rate (calculated from values obtained between 2 and 17 or 24 h post injection of I-125 T3). Figure 2 shows a plot of serum total I-125 radioactivity and T3 associated label between 2 and 17 hours post injection of I-125 labelled T3 from a representitive sheep in Period 2. Animals on the twice maintenance ration had significantly more rapid total I-125 radioactivity disappearance rate (from 2 h post injection of I-125 T3 to the end of the collection in each period), lower

Table 3 Body weight (BW), food intake, serum concentrations of (i) triiodothyronine (T3), (ii) thyroxine (T4) and (iii) iodine (I), and digestibilities of (i) energy, (ii) protein and (iii) dry matter. Potassium iodide was injected intraruminally each day (1.5 mg/d). Data was collected during three separate time periods

Sheep No	Period	Body weight (kg)	Food intake (g/d)	Serum concentrations			Digestibility		
				T4 (ug/100ml)	T3 (ng/100ml)	I (ug/100ml)	E (%)	Ptn (%)	DM (%)
8254	1	38	760	9.36	78.5	23.8	49.6	70.5	50.6
8281	1	32	760	9.21	86.6	28.8	46.8	72.5	46.0
8277	2	32	760	8.33	64.3	21.8	48.9	73.9	47.9
8288	2	32	760	4.57[1,2]	46.1[2]	29.2[2,3]	44.7	74.1	49.9
8290	3	38	760	N.D.[1,2]	89.0[2]	4.5[2,3]	56.0	69.4	57.8
Weighted mean[4]				7.90	68.8	24.6	50.3	71.6	51.7
8254	2	38	1520	7.69	47.5	10.8	39.7	63.4	43.5
8281	2	34	1520	7.84	39.7	19.0	40.3	61.8	38.6
8277	1	38	1520	5.36	67.7	20.5	32.9	67.8	48.4
8288	1	27	1520	1.15[2]	76.0[2]	13.5[2,3]	37.8	66.5	41.1
8240	3	38	1520	13.50[2]	139.0[2]	27.5[2,3]	49.7	63.2	51.0
Weighted mean				5.50	56.5	15.9	41.7	64.3	45.6
S.E.[5]				1.00	4.4	2.1	1.2	0.5	1.5
				NS	NS	*	*	**	NS

NS - not significant; * - $P<0.05$; ** - $P<0.01$

1) T4 was not detectable in serum of sheep 8290

2) These values were not included in the statistical analysis because only two samples were available for analysis, all other concentrations are means from samples collected daily for one week

3) These values were found to be significantly different from the within treatment means (Bliss 1967) and therefore were not used in the statistical analysis

4) Weighted means were calculated by least squares

5) Standard error of the mean

Table 4 Food intake, disappearance rates of (i) triiodothyronine (T3) (ii) total I-125 radioactivity over the same time period as T3 (A) which was 2-24 h or 2-17 h post injection and (iii) total I-125 radioactivity from 2 h post injection of I-125 T3 until the end of sample collection (B) (120-600 h), and distribution volume

Sheep No	Period	Food (g/d)	Disappearance rate (/h) T3	A	B	Distribution volume (% body weight)	
8254	1	760	0.084±.027[1]	0.057±.012	0.0071±.0008	83	120-58[2]
8277	2	760	0.099±.011	0.062±.008	0.0175±.0018	72	89-58
8288	2	760	0.106±.018	0.080±.017	0.0160±.0021	70	98-50
8290	3	760	0.139±.012	0.092±.011	0.0192±.0032	50	70-36
Weighted[3] mean			0.107	0.073	0.0149	69	
8254	2	1520	0.094±.007	0.065±.006	0.0196±.0020	79	90-70
8281	2	1520	0.076±.011	0.077±.009	0.0217±.0024	85	106-68
8277	1	1520	0.081±.053	0.086±.011	0.0079±.0010	148	305-71
8240	3	1520	0.140±.011	0.082±.012	0.0272±.0030	90	140-66
Weighted mean			0.098	0.078	0.0191	100	
S.E.[4]			0.0045	0.0064	0.0034	8	
			NS	NS	*	*	

NS - not significant; * - $P<0.05$

1) x ± standard error

2) Range based on a standard deviation around the extrapolated time zero specific activity of the T3 pool

3) Weighted means were calculated by least square

4) Standard error of the mean

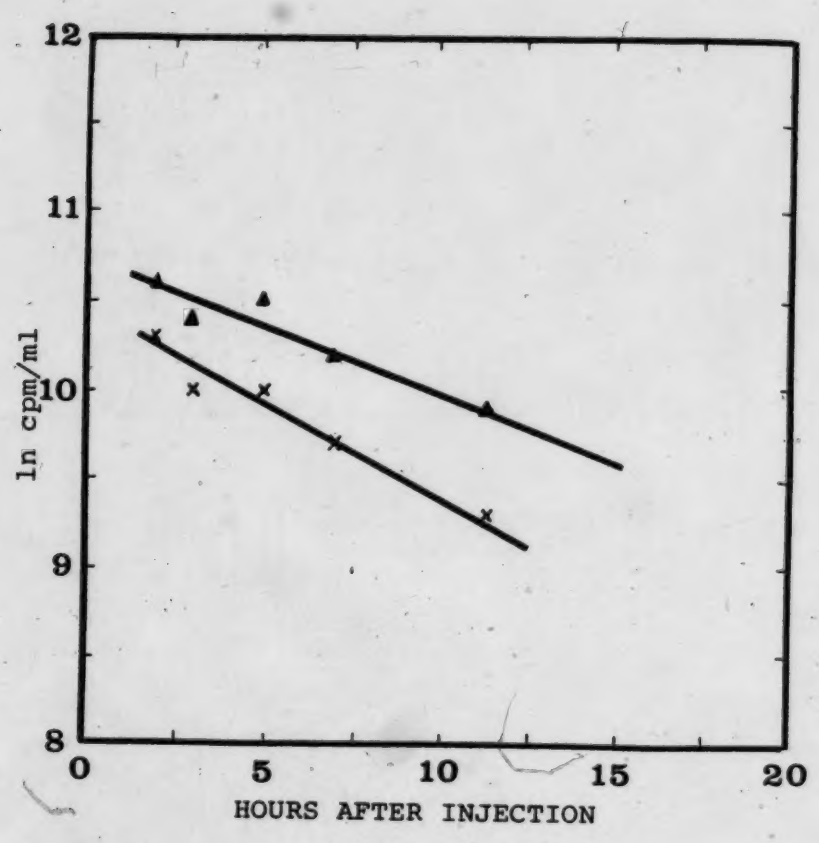


Figure 2 Serum total I-125 radioactivity (▲) and I-125 triiodothyronine radioactivity (x) after an intravenous injection of I-125 triiodothyronine in sheep 8288 during Period 2

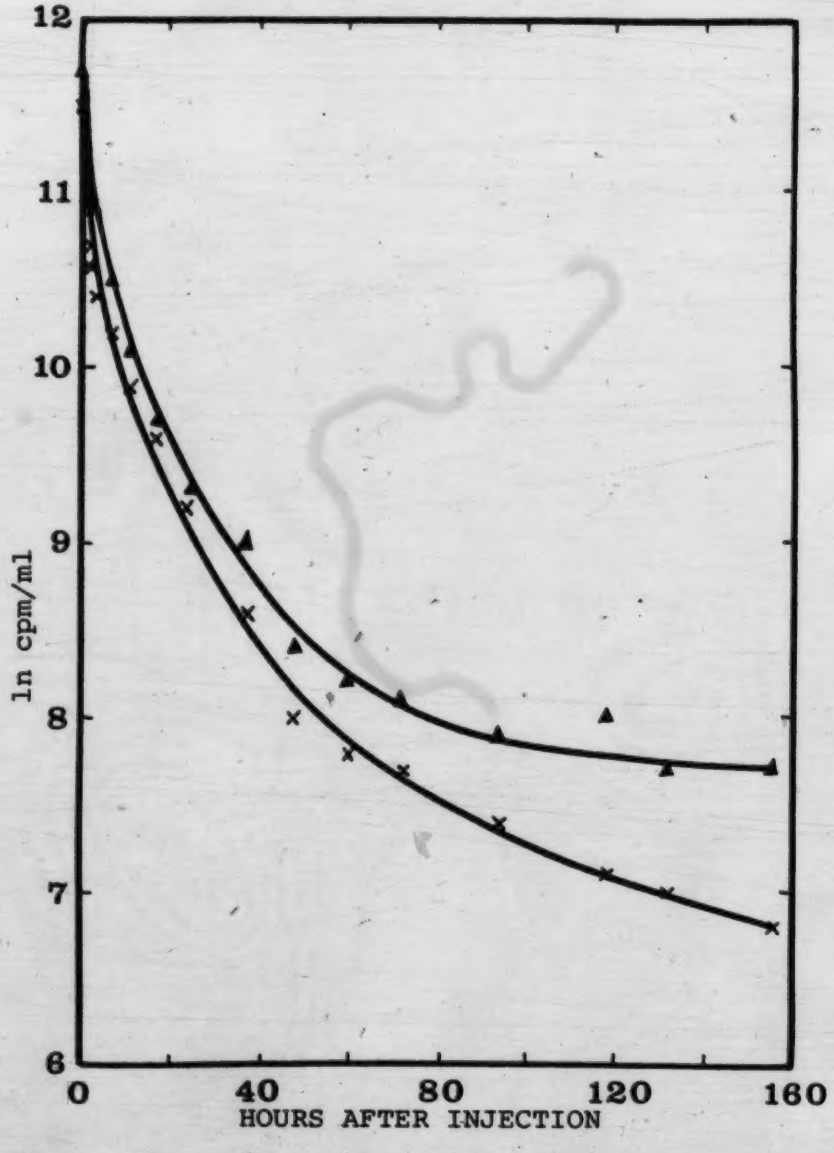


Figure 3 Total serum I-125 radioactivity after an intravenous injection of I-125 triiodothyronine in sheep 8288 which was fed 760 g/d (▲) and sheep 8254 which was fed 1520 g/d (×)

serum iodine and a larger T3 distribution volume (Tables 3 and 4). Digestibility of energy and protein was significantly lower in the sheep on the high level of food intake. From Figure 2 it is apparent that there is a divergence of total I-125 radioactivity and T3 label, although in this data, the difference in the disappearance rate of T3 and total I-125 radioactivity during the first 24 h is not significant.

The disappearance rates of total I-125 radioactivity from the sheep in the two treatment groups were significantly different. Figure 3 shows a plot cf total I-125 radioactivity between 2 and 156 hours post injection in two representitive sheep from Period 2. From this figure it is apparent that first order kinetics do not apply with validity to total I-125 radioactivity. However, first order kinetic analyses were done simply to show that there was a treatment effect on disappearance of total I-125 radioactivity.

Q.2.5 DISCUSSION

When I-125 T3 is metabolized by the body, the products contain I-125. In order to calculate the true disappearance rate of T3 these metabolic products must be removed or the T3 must be isolated. Methods that have been used in the past with I-125 or I-131 labelled T3 or T4 include precipitation of protein with trichloroacetic acid, butanol extraction (this may or may not be followed by alkaline wash) or simply

using total radioactivity of radioactive iodine in unextracted serum or plasma (Chopra et al, 1975; Dussault et al, 1972; Sanchez and Evans, 1972; Evans and Ingram, 1977; Ingram and Kaciuba-Uscilko, 1977). The method used in this experiment has the advantage of being more specific than any of the methods mentioned above. From Figure 1 it can be seen that separation between T3, T4, and iodinated tyrosines was achieved.

Methods such as total I-125 radioactivity in unextracted serum or plasma, trichloroacetic acid precipitation and butanol extraction have the disadvantage of being non-specific as they do not remove metabolites of thyroid hormones. Trichloroacetic acid precipitation and butanol extraction remove most if not all of the iodide, a major metabolite of thyroid hormones. However, other metabolites which may be protein bound are not removed. In these experiments the mean of the disappearance rate of total I-125 radioactivity over the first 24 hours was longer than that of T3. From Figure 2 it is apparent that the curves representing T3 and total I-125 radioactivity are not the same. When this is related to what is known about thyroid hormone metabolism, it is obvious that metabolites of the hormone must be removed or the hormone isolated. This observation is in agreement with the conclusions of Rudolf et al, (1976).

Although in the present study significant treatment effects were seen in the serum iodine concentrations,

caution should be used when extrapolating this to situations where iodine intake is not highly elevated. It is not known if the treatment effects would have been observed if no exogenous iodine had been administered.

T3 distribution volume is a kinetic parameter that varies proportionally with tissue binding of the hormone. It is expressed as a percent of body weight, in this case it corresponds to the equivalent weight of serum that would be required to contain all of the initial injected dose of I-125 T3. Thyroid hormone binding proteins in intracellular and intercellular space have higher affinity for T3 than serum binding proteins (Bernal and Refetoff, 1977). This could account for the large distribution volumes. Dussault et al, (1972) reported that pregnant sheep had T3 distribution volumes of about 60% body weight. Since T3 does not readily cross placental membranes in the maternal to fetal direction (Dussault et al, 1972), pregnant animals would be expected to have smaller distribution volumes for the hormone. The significant differences in distribution volumes seen in our animals may have been caused a change in affinity of tissue T3 binding proteins. It has been reported that the affinity of serum T3 binding proteins can be altered by cold exposure (Cottle and Veress, 1966). If tissue T3 binding proteins can be altered in a similiar fashion this may be a possible explanation for the statistically significant change in the distribution volume.

The observed changes in protein and energy

digestibility confirm that there were changes in digestive function due to the two levels of feed intake. Also, these changes were not accompanied by changes in conventional indices of thyroid status, that is, T3 or T4 concentration or T3 disappearance rate. These digestibility values were low for this type of feed in this lab. Therefore, trials were commenced to determine if high iodide diets decrease the digestibility of feeds. The results were inconclusive for the following reasons. Pregnant sheep were used, the three treatments were 0, 5 and 20 mg KI per day and the length of each period was only 35 days with no time between each treatment period. Cottino et al (1972) reported that three months was insufficient time for radioactive iodine to equilibrate with total iodine in mouse muscle, therefore it is questionable if 35 days is sufficient time for iodine equilibration to be established in sheep. In the experiment conducted for this thesis the sheep were fed KI for 3, 12 or 17 weeks. It is known that placental membranes do actively remove iodine from the maternal circulation (Miller et al, 1975), therefore the fact that these animals were pregnant may be significant. Irvine (1975) cites findings that indicate 5 mg of iodide per day may have a physiologically toxic effect on sheep but he does not indicate what this effect was or the time period involved. It is possible that a long term trial is required to determine if 1.5 mg KI per day can alter digestibility of feed in sheep.

The cause of the period effect on digestibility and

distribution volumes are not known, however, there are some possible explanations. Firstly, the animals in each period had been confined to the metabolic crates and KI treatment for varying lengths of time prior to each period. Some serum samples were lost in Period 1, which may have influenced calculations of disappearance rate and distribution volumes. Finally, the same animals were used in Periods 1 and 2 but not in Period 3.

In summary, under the conditions of the present experiment significant changes could not be detected in concentration or disappearance rate of T3. However, at the tissue level as indicated by T3 distribution volume, there may be changes in T3 binding. This possibility requires further investigation. It was also found that disappearance rate of total I-125 radioactivity measured from 2 h until the end of collection was significantly different in animals on the two levels of feed intake. It is not known what compounds made up total I-125 radioactivity and as mentioned earlier, first order kinetics do not apply. These factors should be taken into account when interpreting this data. The significant differences observed in total I-125 radioactivity disappearance rate may be related to the different serum iodine concentrations. This possibility also requires further investigation.

0.3 CHAPTER 3 : APPEARANCE OF I-125 IN SALIVA, RUMEN FLUID AND FECES

0.3.1 ABSTRACT

Serum, rumen fluid, saliva and feces were collected from sheep at timed intervals after a single injection of I-125 triiodothyronine (T3) into a jugular vein. Total I-125 radioactivity per ml was determined in each sample and compounds containing I-125 were separated by thin layer chromatography and counted.

T3 was not detectable in either rumen fluid or saliva, iodide was the major I-125 containing fraction in both. Total I-125 radioactivity per ml of both rumen fluid and saliva increased to equal to or greater than total I-125 radioactivity per ml serum 4 to 6 hours post injection and total I-125 radioactivity per ml in rumen fluid became near or greater than total I-125 radioactvity per ml serum 10 to 30 hours post injection. This probably indicates that the salivary glands concentrate iodide and release it into the rumen via saliva.

Autoclaving of rumen fluid was found to decrease T3 binding and (or) degradation in rumen fluid *in vitro*. This indicates that the rumen microbiota may have the capacity to metabolize and (or) bind T3.

Iodine-125 labelled T3 was detected in feces but could not be quantified. The fecal excretion of total I-125 varied between animals. However, most of the isotope that was

excreted in the first 72 hours appeared in the feces in the first 24 hours after injection.

0.3.2 INTRODUCTION

According to Slebodzinski (1972) the salivary glands of sheep concentrate and secrete iodide as well as deiodinate triiodothyronine (T3) but it is not known if T3 as such appears in saliva. If T3 is secreted in the saliva it would be an obvious source of this hormone for the rumen. Another source would be direct movement across the rumen wall. Also, it was not known if T3 could be altered or metabolized by the rumen microbiota, consequently, even if T3 did enter the rumen it could be degraded by the microbes at a high rate and therefore be undetectable. Because of this possibility it was necessary to determine if T3 was altered by either microbial or chemical action in the rumen, therefore an *in vitro* experiment was conducted.

Iodine may enter the intestines by one of two routes, firstly as organic iodine from the liver and secondly, as free iodide either from the liver or with the flow of digesta from the abomasum. Thyroid hormones are metabolized and conjugated in the liver. Metabolites, conjugates, and free hormones are secreted into the bile which enters the small intestine. Although conjugated hormones (sulfonate and glucuronate) are not the only iodine containing compounds in the bile, they do make a substantial contribution to the total iodine in the isolated rat liver (Hillier, 1972). The

fate of conjugated and free hormones in the intestines is not known, however, it may be possible for them to be reabsorbed as active hormone (Ramsden, 1977). Iodine in the abomasal flow is almost entirely in the form of free iodide which is readily reabsorbed from the intestines (Miller et al, 1975).

0.3.3 METHODS AND MATERIALS

Saliva, rumen fluid and feces were collected from sheep which were fed 760 or 1520 g/d of alfalfa pellets and had been administered I-125 labelled T3 (see Chapter 2 for details). Serial saliva samples were collected by the methods of Kennedy and Milligan (1978). The saliva was allowed to sit for a few minutes until food particles settled, the supernatant was then removed and stored at -20 C until analyses. Rumen fluid was collected with a 50 ml syringe that was attached to a piece of vinyl tubing (30 cm long) which was inserted through the fistula. The rumen fluid was centrifuged at 27,000xg for 15 minutes, the supernatant was then removed and stored at -20 C until analyses. Analyses for both rumen fluid and saliva consisted of determination of total radioactivity and thin layer chromatography to identify labelled fractions.

For the in-vitro experiment, rumen fluid from a rumen fistulated sheep that was not part of the previously described study (fed 500 g brome hay plus 50 g glucosyl urea twice each day) was used. Rumen fluid was collected as

described previously and strained through a single layer of cheesecloth into an insulated flask. Exposure of the rumen fluid to air was kept to a minimum, the head gas in the flask was displaced with nitrogen gas for transportation to the laboratory. In the laboratory, the rumen fluid was anaerobically diluted (1:1) with reduced (sufficient sodium dithionite to reduce resazurin, a redox indicator) phosphate buffer (pH 6.5, 0.2 M) containing sufficient maltose and glucose to give a final concentration of 0.25% of each. Ten milliliter aliquots were dispensed into 40 ml screw cap tubes. One-half of the tubes were autoclaved at 110 C for 20 minutes. Labelled T3 (1091 uCi/ug, 90% pure) was added to all tubes in sufficient amounts to provide 7500 cpm per milliliter. Incubation was carried out in duplicate at 38 C for 0, 0.33, 0.66, 1, 1.5, 2, and 2.5 hours. After incubation a drop of chloroform was added to each tube and they were immediately centrifuged at 27,000xg for 15 minutes. The supernatants were removed and stored at -20 C until chromatography was done.

Saliva was chromatographed in the same manner as was serum (see Chapter 2). Rumen fluid and samples from the <u>in-vitro</u> experiment were chromatographed directly. The method used was to add 15 ug each of T3, T4 and KI to a 0.10 ml sample. This solution was applied directly to a TLC plate. For some samples it was necessary to use less than 0.10 ml of rumen fluid because of large amounts of dissolved or suspended material in the sample which caused poor

separation. TLC plates and solvents have been described in Chapter 2.

Fecal samples were mixed and 1.0 g subsamples were obtained for analyses of labelled compounds. Total I-125 radioactivity was determined in wet samples that had been crushed and mixed. Dried samples were not used to avoid generation of I-125 contaminated dust during grinding. Labelled fractions in the feces were extracted from 1.0 g of wet sample by washing it once with 5 ml of phosphate buffer (pH 6.5, 0.2 M) containing 0.1% Triton X100 and then centrifuging the mixture at 27,000xg for 10 minutes. This procedure was repeated two more times using 3 ml of the buffer solution after which the supernatants were pooled. This method of extraction removed about 30% of the total I-125 radioactivity from feces. The unextracted labelled compounds were not identified. The pooled extracts were chromatographed in the same manner as rumen fluid.

0.3.4 RESULTS

There was no detectable T3 in either rumen fluid or saliva. Most of the label in these samples chromatographed as iodide although rumen fluid samples did not have sufficient radioactivity for accurate quantitative analyses. Total radioactivity and iodide associated radioactivity for saliva and rumen fluid are reported in Appendices 2 and 3. As can be seen from Figure 4 and Appendices 1 and 2, serum total radioactivity was consistently lower than was saliva

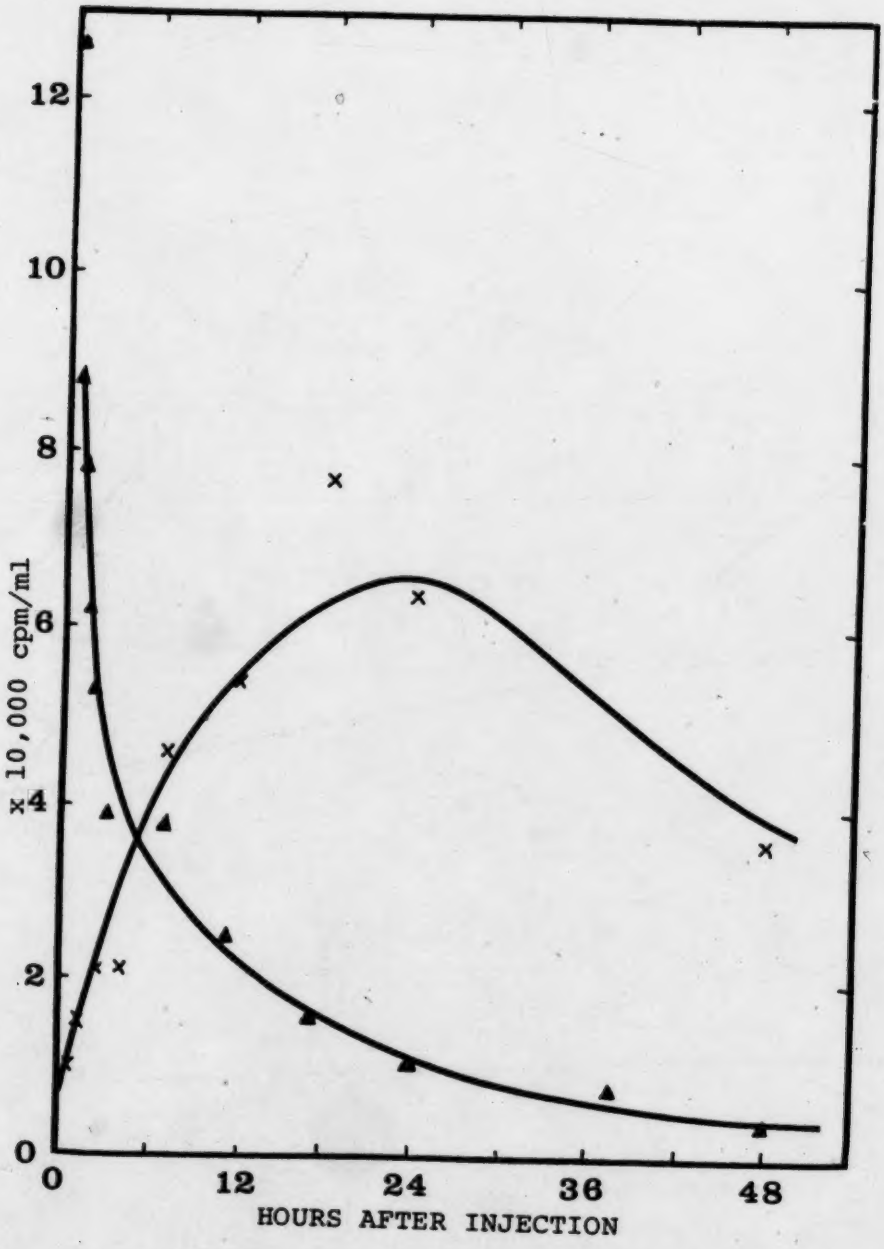


Figure 4 Saliva (x) and serum (▲) total I-125 radioactivity after injection of L-125 triiodothyronine in sheep 8288 during Period 2

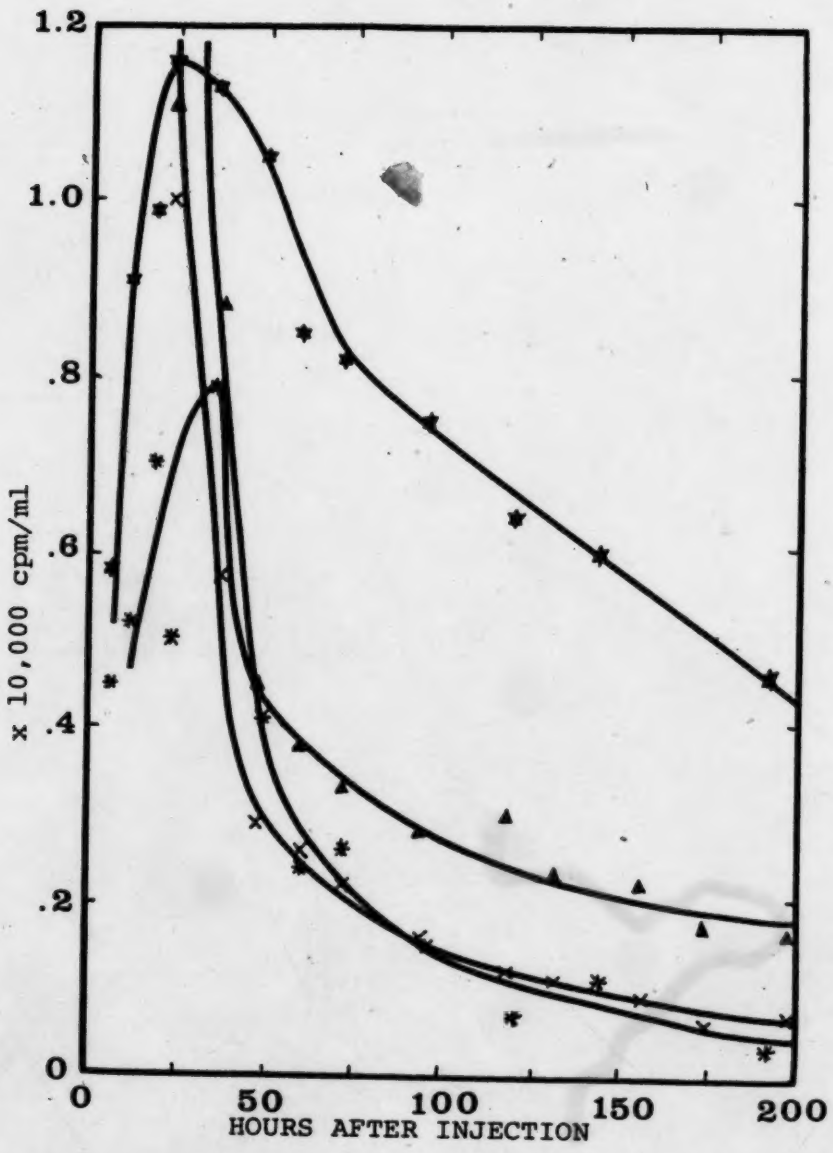


Figure 5 Rumen fluid total I-125 radioactivity from sheep 8288 which was fed 760 g/d (*) and sheep 8254 which was fed 1520 g/d (*) and serum total I-125 radioactivity from sheep 8288 (▲) and 8254 (x) after an intravenous injection of I-125 triiodothyronine during Period 2

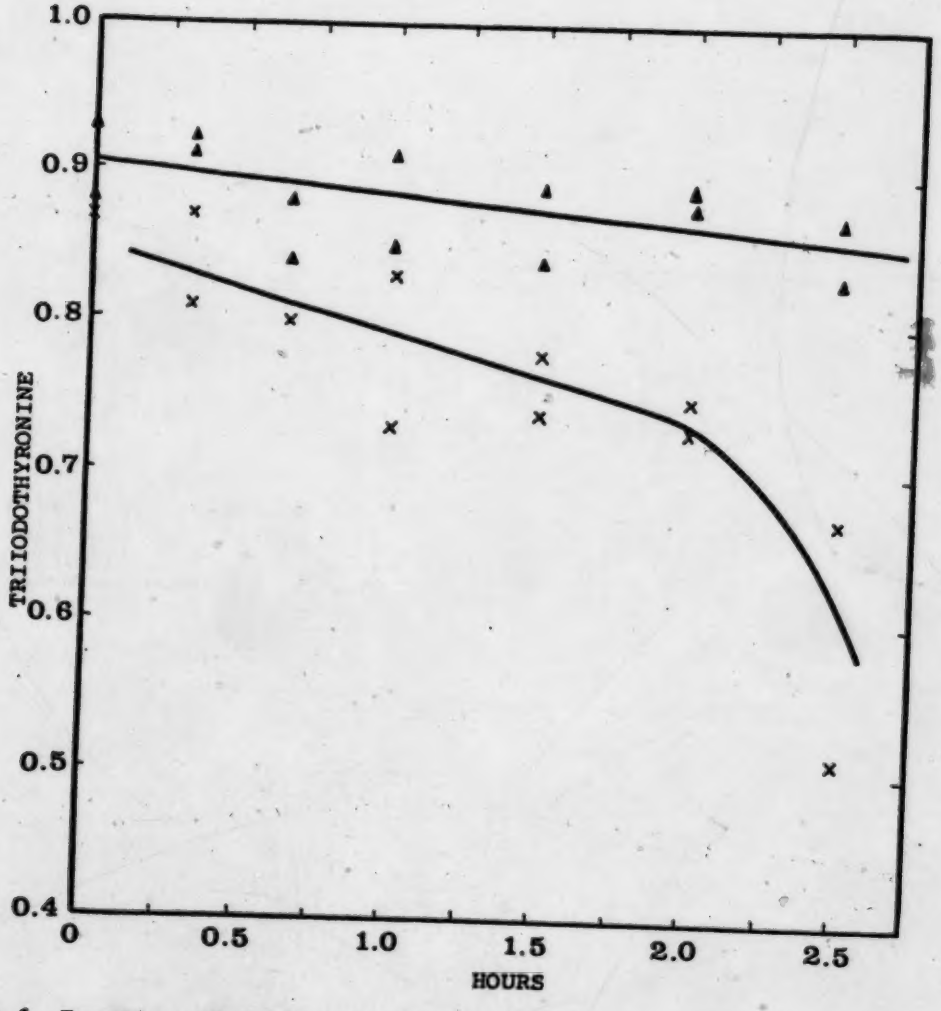


Figure 6 Fraction of I-125 triiodothyronine in autoclaved (▲) and unautoclaved (x) rumen fluid during an <u>in vitro</u> experiment

total radioactivity after an initial equilibration period (0-6 h). Total radioactivity in rumen fluid also was near or higher than that of serum after a period of 10-30 h. It also appeared as though total radioactivity was more concentrated in the rumen of the sheep receiving the maintenance ration than in the rumen of the sheep recieving the twice maintenance ration. This is illustrated graphically in Figure 5 using representative sheep from each treatment group during Period 2.

From Figure 6 it can be seen that unautoclaved rumen fluid has the ability to alter T3. A paired t-test showed that the fraction of T3 at time 0 in the autcclaved rumen fluid was significantly ($P>0.05$) greater than in the unautoclaved rumen fluid. This indicates that there is some factor in rumen fluid that prevents a fraction of the T3 from migrating as T3 and this factor is destroyed by autoclaving. This may have been due to binding or metabolism of T3 in the rumen fluid.

The excretion of total I-125 radioactivity in feces during the first 72 hours after injection of the hormone was highly variable (range 6.8% to 89.7%, average 47.6% the total dose. (See Table 5 and Appendix 4). Most (>60%) of the label that was excreted in feces during the first 72 h appeared in the first 24 h. No statistical analyses were performed because of the variability between animals and periods.

The extraction method was too inefficient to allow

Table 5 Accumulation of I-125 in feces from sheep fed alfalfa pellets at 760 g/d or 1520 g/d, 1.5 mg KI was injected intraruminally each day. I-125 was injected intravenously at time zero as 1,100,000,000 cpm I-125 triiodothyronine of 90% purity

Sheep No	Feeding level (g)	Accumulated counts (x 10,000,000 cpm) 0 - 24 h	0 - 50 h	0 - 72 h
8254	760	29.9[1]	35.1[2]	37.5
	1520	58.5	73.5	75.3
8277	760	36.3	53.1	55.4
	1520	47.5[1]	51.3[2]	55.1
8288	760	86.2	97.4	99.7
8281	1520	57.2	73.6	76.0
8290	760	10.1	12.5	13.7
8240	1520	5.1	7.3	7.5

1) Actual time was 0-27 h

2) Actual time was 0-45 h

quantitative determination of fecal excretion of labelled compounds. However, iodide and T3 were both present.

0.3.5 DISCUSSION

Total radioactivity in the rumen did not reach a maximum until 10 to 30 h after injection of the labelled hormone. Because of this, much of the labelled hormone may have disappeared from the blood pool before it had a chance to enter the rumen.

Slebodzinski, (1972) and Miller et al,(1975) indicate that salivary glands can concentrate iodine from serum. This is also indicated by the finding that saliva total radioactivity was higher than serum total radioactivity and iodide was the major I-125 containing fraction in saliva. Also, saliva was probably the source of rumen iodine since the wall rumen does not actively transport or concentrate iodide (Miller et al, 1975).

It appears as though T3 is altered or bound by unautoclaved rumen fluid. Even though viable rumen microbes would appear to be a logical cause of these alterations, the present data cannot be used to eliminate the possibility of non-microbial chemical action which was prevented by autoclaving.

No evidence of T3 in the saliva was found, therefore if T3 does get into the rumen it would have to pass through the rumen wall. However, binding of T3 to blood proteins would limit passive diffusion. Microbes attached to the rumen wall

could metabolize the hormone. It would then be undetectable by the methods used even though it may be functionally present.

Even if T3 can be metabolized in the rumen this does not mean that it does have an effect on rumen microbe metabolism. This point requires further investigation.

There is no obvious reason for the large variation in total I-125 radioactivity excretion, however, one possibility relates to the high iodine diet that these sheep were fed. In these experiments the sheep in Period 1 had been receiving the high iodine diet for 12 weeks, in Period 2 they had received the iodine for 17 weeks and in Period 3 they had received the iodine for 3 weeks. Consequently, the sheep in the three periods may have been at different states of iodire equilibration (Cottino et al, 1972).

0.4 CHAPTER 4 : METABOLITES OF TRIIODOTHYRONINE IN SHEEP URINE

0.4.1 ABSTRACT

A tracer dose of I-125 labelled triiodothyronine was injected into a jugular vein of six yearling ewes. Urine was collected by bladder catheter for 72 hours. Labelled organic compounds were extracted with acidified t-amyl-alcohol and separated by thin layer chromatography on silica gel.

Labelled triiodothyronine (T3), iodide, and an unidentified fraction appeared in the urine within 2 hours of injection of the labelled T3. On the basis of chromatography, the unknown was not 3,3'-diiodothyronine, 3,5-diiodothyronine or triiodothyroacetic acid. However, the possibility of the unknown being mono- and diiodotyrosines of conjugates of T3 could not be eliminated. The unknown did not reach detectable levels in the serum of the sheep. This indicates that the unknown is either produced or concentrated by the kidneys. During the first 12 hours following administration of the labelled T3, 0.82% of the initial dose of T3 appeared as extractable T3, and 6.20% appeared in the urine as extractable unknown.

0.4.2 INTRODUCTION

Most work on urinary excretion of thyroid hormone metabolites has been done with laboratory species and humans (Shakespear and Burke, 1976). No reports on urinary

excretion of thyroid hormones from sheep have been found.

Products of triiodothyronine (T3) metabolism in lab species and humans include: 3,3'-diiodothyronine, 3',3-diiodothyronine (3',3-T2), 3',3,5-triiodothyroacetic acid (TRIAC) and 3',3,5-triiodcthyropropionic acid (TRIPOP). In humans and rats T3 and 3',5',3-triiodothyronine (rT3) are major sources of 3',3-T2 (Hufner and Grussendorf, 1978; Chopra et al 1978; Gavin et al 1978). The 3',3-T2 is rapidly deiodinated and excreted in urine (Stanbury and Morris 1957). The deamination and decarboxylation products TRIAC and TRIPOP are also produced in humans (Nakamura et al,1978). Deamination of thyroid hormones is enzymatic in nature and controlled by the alpha-keto acid substrate. In the presence of pyruvate or oxaloacetate, the deiodination enzyme (from rabbits) is specific for T3. In the presence of alpha keto glutarate, the enzyme also deaminates 3,5-dinitrothyroxine (Soffer et al, 1973). Conjugates of T3 and thyroxine (T4) are produced in the kidneys. These conjugates of T3 make up 50% of the total T3 in human urine (Shakespear and Burke, 1976; Burke and Shakespear, 1976) and may be present in pregnant sheep serum (Dussault et al, 1972). Splitting of the ether bond of T4 has been reported but it is unclear whether the T4 molecule or a metabolite of it is cleaved (Wynn and Gibbs, 1964).

0.4.3 METHODS AND MATERIALS

Six sheep that were fed a near maintenance (760 g/d) or

twice maintenance (1520 g/d) ration of alalfa pellets were given a single injection of I-125 labelled T3 (see Chapter 2 for details). Urine samples collected from the sheep during Period 1 were used only for analysis of total radioactivity of I-125. This was due to differences in the collection schedule in this period and the lack of inclusion of a suitable antimicrobial agent. Urine samples were collected by bladder catheter and preserved with 0.02% sodium azide. Volumes were recorded and subsamples were stored at -20 C until laboratory analyses could be performed.

Total radioactivity was determined and labelled compounds were extracted from urine by the following procedure: (i) 0.0003 g of T3, T4 and KI (each dissolved in 0.10 ml 0.04 N NaOH) were added to 0.50 ml of urine and mixed on a vortex mixer for 15 seconds, samples were cooled to 0 C for 15 minutes; (ii) 0.80 ml of t-amyl alcohol (saturated with 10% sulfuric acid) was added and mixed for 60 seconds on a vortex mixer; (iii) The t-amyl alcohol and aqueous fractions were separated by centrifuging at 1100xg for 5 minutes, the t-amyl alcohol layer was removed; (iv) The aqueous layers were re-extracted and the extracts were pooled, 0.10 ml of the pooled extracted was chrcmatographed on silica gel G plates (see Chapter 2). This method was capable of detecting 4000 cpm in one milliter of urine (with an accuracy of at least 7%) of either T3 or extractable unknown. Total urinary excretion of radioactive compounds was calculated from total urine volume and the amount of

radioactivity per ml. The error (of the estimated T3 or extractable unknown excreted) would be expected to increase with time after injection of the labelled hormone because the specific activity of the urine decreased (resulting in less accurate counting).

Preliminary tests showed that this method of extraction removed more than 98% of a tracer amount of labelled T3 from either urine or distilled water. Extraction of iodide was found to be variable and less efficient, therefore extractable iodide is not reported. The efficiency and consistency of extraction of the unknown could not be determined, however the amount extracted in relation to T3 was substantial (Table 6).

The Rf (relative fraction of migration) of diiodotyrosine (DIT), monciodotyrosine (MIT), thyroxine (T4), 3,5-diiodothyronine (3,5-T2), 3',3,5-triiodothyroacetic acid (TRIAC), T3 and potassium iodide were also determined.

0.4.4 RESULTS

An unidentified I-125 containing fraction accounted for a large portion of the total extractable I-125 radioactivity excreted in the urine. As can be seen from Table 6, the extractable unknown accounted for the a large proportion of the total I-125 radioactivity excreted during the first 18 h after injection of the labelled hormone. T3 and iodide were also present, however, T3 was a minor component and the

contribution of iodide could not be accurately determined. From Figure 7 it can be seen that as time progressed, the fraction of total extractable label in urine decreased until 36 h post injection. Also, during the first 24 h the unknown fraction decreased and became undetectable. After 12 h, T3 was undetectable. The accumulation of excreted unknown and T3 is illustrated graphically in Figure 8. This figure shows that about 9% (90,000,000 cpm) of the initial dose was excreted as the unknown in urine within 24 h. This was considerably more than the portion of the injected T3 that was excreted unchanged. Table 7 is a tabulation of the excretion of total I-125 radioactivity in urine. It is evident that the excretion of total I-125 is highly variable between animals. Also more I-125 was excreted during the first 24 h period than in the second or third 24 h period after injection of the labelled hormone.

From the Rf values in Table 8 it can be seen that iodide migrates by itself free from iodinated thyronines. T3 migrates at the same rate as does 3,5-T2, consequently, 3,5-T2 may be mistakenly identified as T3 in this system. However, it has not been reported as a major metabolite of T3 in any species, therefore interference from the compound was considered to be unlikely. 3',3-T2 was not tested in this system, however, Latham et al, (1976) reported that when they used the same conditions, 3',3-T2 migrated between T3 and T4. Significant counts were not found in this area. The unknown migrated more slowly than T4 in the area that

Table 6 Mean (±S.D.) hourly urinary excretion rate of total I-125 radioactivity, extractable unknown and extractable triiodothyronine (T3) from sheep fed 760 g/d or 1520 g/d alfalfa pellets (treatments were combined, 3 sheep in each) 0-72 hours after intravenous injection of 1,100,000,000 cpm I-125 T3 (90% pure)

	Rate of excretion of I-125 (x 10,000,000 cpm/h)		
Time (h)	Total	Unknown	T3
0-2	0.54 ± 0.36	0.30 ± 0.21	0.09 ± 0.03
2-4	2.07 ± 2.18	1.05 ± 0.78	0.16 ± 0.06
8-12	0.85 ± 0.76	0.42 ± 0.31	0.03 ± 0.01
12-28	0.55 ± 0.39	0.26 ± 0.14	
18-24	0.24 ± 0.19	0.09 ± 0.06	
24-36	0.19 ± 0.17	0.05 ± 0.04	
36-50	0.13 ± 0.18		
50-60	0.08 ± 0.08		
60-72	0.10 ± 0.15		

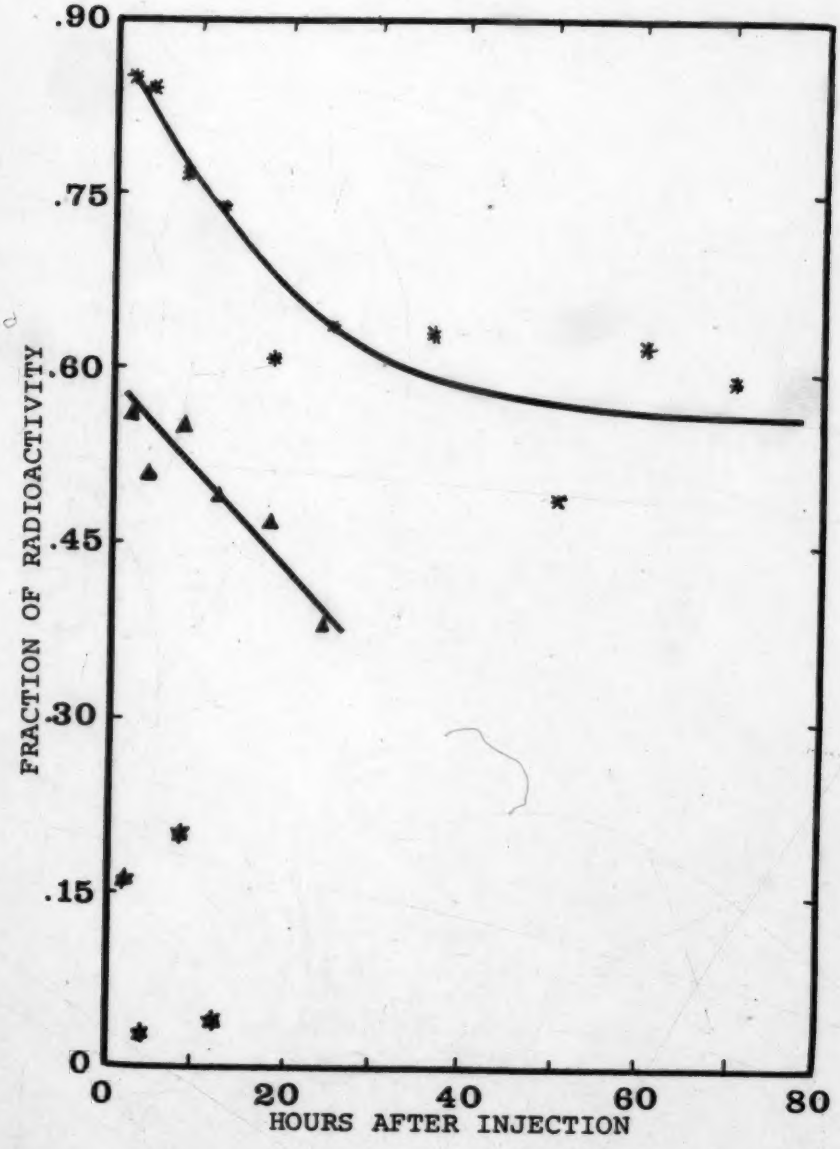


Figure 7 Occurrance of extractable I-125 radioactivity (*), extractable unknown (▲) and extractable triiodothyronine (*) in urine of sheep after an intravenous injection of I-125 triiodothyronine

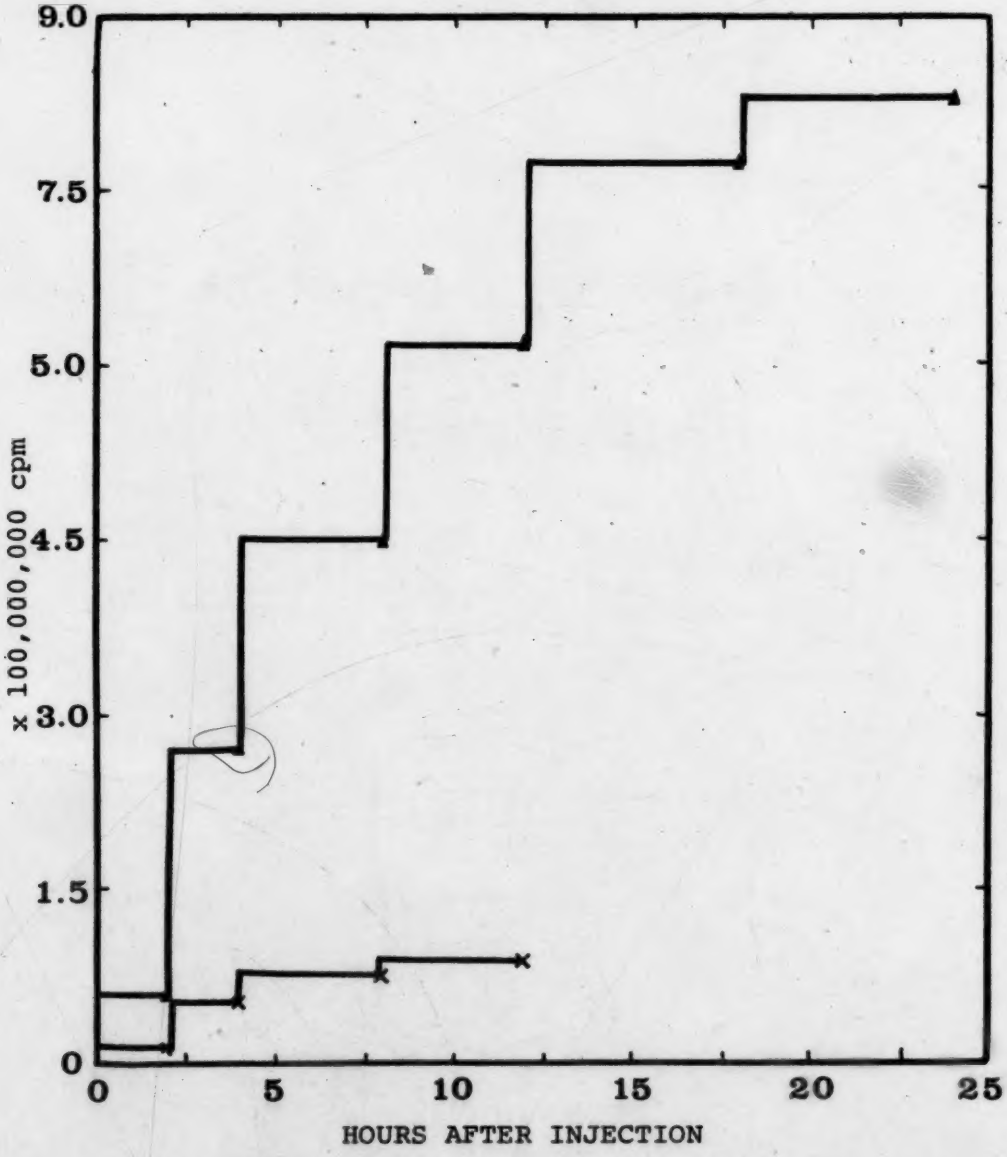


Figure 8 Accumulation of extractable unknown (▲) and triiodothyronine (x) in the urine of sheep after an intravenous injection of I-125 triiodothyronine at time zero

Table 7 Accumulation of I-125 in urine from sheep fed alfalfa pellets at 760 g/d or 1520 g/d, 1.5 mg KI was injected intraruminally each day. I-125 was injected intravenously at time zero as 1,100,000,000 cpm I-125 of 90% purity

Sheep No.	Feeding Level g/d	Accumulated counts (x 10,000,000 cpm)		
		0-24h	0-50h	0-72h
8254	760	3.4[1]	4.4[2]	5.5
	1520	6.2	7.3	8.0
8277	760	14.7	17.4	18.2
	1520	7.5[1]	8.1[2]	8.6
8288	760	10.9	12.1	12.6
8281	1520	10.2	13.1	14.0
8290	760	42.2	55.6	62.8
8240	1520	16.2	19.9	21.7

1) Actual time was 0-27 h

2) Actual time was 0-50 h

Table 8 Relative fraction of migration (Rf) of diiodotyrosine (DIT), monoiodotyrosine (MIT), thyroxine (T4), 3,5-diiodothyronine (3,5-T2), 3',3,5-triiodothyronine (T3), 3',3,5-triiodothyroacetic acid (TRIAC), potassium iodide (I) and unknown (U) on silica gel G thin layer chromatography plates

Compound	Rf
DIT	0.16
MIT	0.14
T4	0.26
3,5-T2	0.37
T3	0.36
TRIAC	0.56
I	0.64
U	0.15

corresponded to the iodinated tyrosines.

0.4.5 DISCUSSION

Urine samples could not be chromatographed without extraction because the Rf values were found to be highly variable between samples. This could have resulted from variable concentrations of salts and (or) buffering capacity of the urine samples. Also, organic compounds in the unextracted urine made detection of the T3 and T4 bands difficult, therefore, labelled compounds were extracted into acidified t-amyl alcohol. No evidence of degradation of T3 during the extraction process was found during verification of the method. It was also found that T3 was extracted to a greater extent than was iodide.

On the basis of chromatography, the unknown fraction could have been iodinated tyrosines. However, the sulfonic and glucuronic acid conjugates of T3 could not be ruled out (Shakespear and Burke, 1976; Burke and Shakespear, 1976; Dussault *et al*, 1972). Shakespear and Burke (1976) reported that conjugates account for about 50% of the total T3 present in human urine. The unknown in this study accounted for considerably more than 50% of the sum of the unknown and T3. However, species and diet differences must be taken into account when extrapolating to sheep.

The sulfonic and gluconuric acid conjugates of T3 are chemically unstable and are cleaved by acidic conditions and possibly alkaline conditions (Shakespear and Burke, 1976).

Both of these conditions occurred during the extraction and chromatography procedures. However, since the conjugates are difficult to prepare and obtain in pure form (Shakespear and Burke, 1976) it was not determined if the conjugates were extracted or stable in the procedures used, therefore it was assumed that they would be extracted intact. Molecules of the size and polarity of these conjugates could be expected to migrate in the region of the unknown (T. Fenton and J.R. Thompson, personal communication).

The unknown that Dussault et al (1972) found in blood of pregnant sheep was thought to be a conjugate of T3. Although precise figures were not given, it was implied that equal amounts of the unknown and T3 appeared in maternal serum after I-131 administration to the fetus. The unknown in this study did not appear in serum in sufficient amounts for quantitative determination.

Monoiodotyrosine and diiodotyrosine may be produced from T4 and T3. Wynn and Gibbs (1964) showed that the ether bond in T4 could be cleaved by rat liver microsomes. It is unclear whether the molecule cleaved was actually T4 or a derivative of it such as T3 or 3',3-T2. Therefore, the possibility that other thyronines are not cleaved cannot be ruled out. Cleavage of any of the thyronines would produce a molecule of iodinated tyrosines plus a 1,4 hydroquinone derivative (Wynn and Gibbs, 1966). Because there are several compounds that could be produced by the animal that might migrate to the area of the unkown, its identity cannot be

suggested with any certainty.

0.5 CHAPTER 5 :GENERAL CCNCIUSIONS AND DISCUSSION

0.5.1 KINETICS AND DISTRIBUTION

The data presented in this thesis indicate that either a maintenance or twice maintenance ration does not affect the circulating concentration of triiodothyronine (T3) or its disappearance rate. These two levels of feed intake did cause alterations in the distribution volume of T3, which presumably occurred through a change in affinity cr number of binding sites.

0.5.2 SALIVA, RUMEN FLUID AND FECES

In this study T3 was not detectable in either saliva or rumen fluid. In vitro, there appears to be some action of the rumen micrcbicta on T3. It was not determined what this action was but it may have been due to binding followed by metabolism.

It is possible that recycling of iodide through the alimentary tract contributed to the differences observed in total I-125 radioactivity disappearance rate. However, the present data is insufficient to prove this.

T3 was present in feces tut the amount could not be accurately quantified. If T3 does influence the metabolism of microbes in the gastrointestinal tract of sheep, the hormone present in the hind gut may be of importance.

0.5.3 URINARY EXCRETION PRODUCTS OF TRIIODOTHYRONINE

A large unidentified fraction of T3 metabolite(s) was detected in the urine of the sheep. Some compounds which could make up all or part of this fraction are mono- or diiodotyrosine and sulfonic or glucuronic acid conjugates of T3. The unknown fraction accounted for more of the total I-125 radioactivity excreted in the urine than did T3. In monogastrics there have been no reports of the possible products mentioned above being present in such large quantities. Thus it may be possible that thyroid hormone metabolism in ruminants and monogastrics is different. It will be necessary to chemically define the unknown fraction before any conclusions can be reached. As in all other phases of this experiment the possibility that the high level of iodine fed to the sheep may alter T3 metabolism must be kept in mind.

0.6 BIBLIOGRAPHY

ABDULLAH, R. and FALCONER ,I.R. 1977. Responses of thyroid activity to feed restriction in the goat. Aust. J. Biol. Sci. 30:207-215.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1975. Methods of Analysis. A.O.A.C. Washington D.C.

AZIZI, F. 1978. Effect of dietary composition on fasting induced changes in serum thyroid hormones and thyrotropin. Metab. 27:935-942.

BERNAL, J. and REFETOFF, S. 1977. The action of thyroid hormones. Clin. Endocrinol. 6:227-249.

BISWAS, S.K. 1975. Effects of thyroxine on bacterial growth *in-vitro*. Lancet 2:717-716.

BLISS, C.I. 1967. Statistics in Biology. Volume One. McGraw-Hill Book Company, New York, St. Louis, San Francisco, Toronto, London, Sydney.

BURKE, C.W. and SHAKESPEAR, R.A. 1976. Triiodothyronine and thyroxine in urine. II. Renal handling, and effect of urinary protein. J. Clin. Endocrinol. Metab. 42:504-513.

CALBIOCHEM, 1975. Doc. No. 2019. Calbiochem La Jolla, Ca. U.S.A.

CHOPRA, I.J.,HO, R.S. and LAM, R. 1972. An improved radioimmunoassay of triiodothyronine in serum: Its application to clinical and physiological studies. J. Lab. Clin. Med. 80:729-739.

CHOPRA, I.J., SACK, J. and FISHER, D.A. 1975. 3,3',5'-triiodothyronine (reverse T3) and 3,3',5-triiodothyronine (T3) in fetal and adult sheep: Studies of metabolic clearance rates, production rates, serum binding and thyroidal content relative to thyroxine. Endocrinolcgy 97:1080-1088.

CHOPRA, I.J., WU, S.Y., NAKAMURA, Y. and SCLCMCN, D.H. 1978. Monodeiodenation of 3,5,3'-triiodothyronine and 3,3',5'-triiodothyronine to 3,3'-diiodothyronine in vitro. Endocrinology 102:1099-1106.

CHRISTOPHERSCN, R.J. 1976. Effects of prolonged cold and the outdoor environment an apparent digestibility in sheep and cattle. Can. J. Anim. Sci. 56:201-212

CCTTINC,F., CARLI, M., MAGRC, G., ZOPPETTI, G. and CCSTA, A. 1972. An investigation on the concentration and nature of skeletal muscle iodine compounds. J. Nucl. Biol. Med.

16:39-43.

COTTLE, W.H. and VERESS, A.T. 1966. Serum binding and biliary clearance of triiodothyronine in cold-acclimated rats. Can. J. Physiol. Pharmcol. 44:571-574.

DUSSAULT, J.H., HOBEL, C.J., DISTEFANO, J.J.III, ERENBERG, A. and FISHER, D.A. 1972. Triicdothyronine turnover in maternal and fetal sheep. Endocrinology 90:1301-1308.

EKHOLM, R. 1977. Thyroid hormone secretion. 91-110. "First European Symposium on Hormones and Cell Regulation." Editors Dumont, J. and Nunez, J. Elsevier/North Holland Biomedical Press. New York.

EVANS, S.E. and INGRAM, D.L. 1977. The effect of ambient temperature upon the secretion of thyroxine in the young pig. J.Physiol. 264:511-521.

GAVIN, L.A., HAMMOND, M.E., CASTLE, J.N. and CAVALIERI, R.R. 1978. 3,3'-diiodcthyronine production, a major pathway of perpheral iodothyronine metabolism in man. J. Clin. Invest. 61:1276-1285.

HARLAND, W.A. and ORR, J.A. Editors. 1975. Thyroid Hormone Metabolism. Academic Press. New York and London.

HILLIER, A.P. 1972. Active secretion of thyroxine into the bile: The role of tissue thyroxine-binding sites. J. Physiol. 221:459-470.

HUFNER, M. and GRUSENDORF, M. 1978. Investigation on the deiodination of thyroxine (T4) to 3,3'-diiodothyronine (3'3-T2) in rat liver homogenate. Clin. Chim. Acta. 85:243-251.

HYCEL INC. 1974. For the quantitative determinatcn of protein bound iodine in serum. Hycel Inc. Lit. No. 5764. Houston.

INGRAM, D.L. and KACIUBA-USCILKO, H. 1977. The influence of feed intake and ambient temperature on the rate of thyroxine utilization. J. Physiol. 270:431-438.

IRVINE, C.H.G. 1975. Four compartment model of thyroxine metabolism. 163-188. "Thyroid Hormone Metabolism." Edited by Harland, W. A. and Orr, J.A. Academic Press. New York and London.

IRVINE, C.H.G. 1974. Concentration of thyroxine in cellular and extracellular tissues of the sheep and the rate of equilibration of labelled thyroxine. Endocrinology 94:1060-1071.

KENNEDY, P.M. and MILLIGAN, L.P. 1978. Effects of cold exposure on digestion, microbial synthesis and nitrogen transformation in sheep. Br. J. Nutr. 39:105-117.

KENNEDY, P.M., YOUNG, B.A. and CHRISTOPHERSON, R.J. 1977. Studies on the relationship between thyroid function, cold aclimation and retention time of digesta in sheep. J. Anim. Sci. 45:1084-1089.

LATHAM, K.R., RING, J.C. and BAXTER, J.D. 1976. Solubilized nuclear "receptors" for thyroid hormones. J. Biol. Chem. 251:7388-7397.

LEE, P.A., WALLIN, J.D., KAPLOWITZ, N., BURKHARTSMEIER, G.L., KANE, J.P. and LEWIS, S.E. 1977. Endocrine and metabolic alterations with food and water deprivation. Am. J. Clin. Nutr. 30:1953-1962.

LEVIN, R.J. 1969. The effect of hormones on the absorptive, metabolic and digestive functions of the small intestine. J. Endocr. 45:315-348.

McGUIRE, R.A. and BERMAN, M. 1978. Maternal, fetal and amniotic fluid transport of thyroxine, triiodothyronine, and iodide in sheep: A kinetic model. Endocrinology. 103:567-576.

MERCK MANNUAL, 12th edition. Edited by D.N. Holvey. Merck Sharpe and Dohme Research Laboratories. Rahway N.J. 1972.

MILLER, J.K., SWANSON, E.W., LYKE, W.A., MCSS, B.R. and BYRNE, W.F. 1974. Effects of thyroid status on digestive tract fill and flow rate of undigested residues in cattle. J. Dairy Sci. 57:194-197.

MILLER, J.K., SWANSON, E.W. and SPALDING , G.E. 1975. Iodine absorption excretion, recycling, and tissue distribution in the dairy cow. J. Dairy Sci. 58:1578-1593.

NAKAMURA, Y.,CHOPRA, I.J. and SOLOMON, D.H. 1978. An assessment of the concentration of acetic acid derivatives of 3,5,3'-triiodothyronine in human serum. J. Clin. Endocrinol. Metab. 46:91-97.

NATIONAL ACADEMY OF SCIENCES. 1975. Nutrient requirements of sheep. Fifth Revised Edition. National Academy of Sciences. Washington D.C.

O'CONNOR, J.J., MYERS, G.S.Jr, MAPLESDEN, D.C. and VANDER NOOT, G.W. 1970. Chemical additives in rumen fermentations: In vitro effects of various drugs on rumen volatile fatty acids and protozoa. J. Anim. Sci. 30:812-818.

OPPENHEIMER, J.H., SCHWARTZ, H.L., SURKS, M.I., KOERNER,

D.H. and DILLMAN, W. 1975. Physiological significance of nuclear receptor sites. 189-199. In "Thyroid Hormone Metabolism" edited by W.A. Harland and J.S. Orr. Academic Press, 1975. New York and London.

PATEL, Y.C. and BURGER, H.G. 1973. A simplified radioimmunoassay for triiodothyronine. J. Clin. Endocrinol. Metab. 36:187-190.

PINCUS, S.H. and KLEBANOFF, S.J. 1971. Quantitative leukocyte iodination. N. Engl. J. Med. 284: 744-750.

RAMSDEN, D.B. 1977. Peripheral Metabolism and Action of Thyroid Hormones. Eden Press, Montreal and Lancaster.

ROGOWSKI, P., SIERSBAEK-NIELSEN, K. and HANSEN, J.M. 1978. Urinary excretion of thyroxine and triiodothyronine in different thyroid function states in man. Acta Endocr. 87:525-534.

RUDOLF, M., SAKURADA, T., VAGENAKIS, A., FANG, S., BRAVERMAN, L. and INGBAR, S. 1976. Demonstration of sequential monodeiodination as the major pathway of iodothyronine metabolism. Clin. Res. 24:429A.

SANCHEZ, O. and EVANS, J.W. 1972. Ovine thyroid adaptation to heat exposure. Environ. Physiol. Biochem. 2:201-208.

SHAKESPEAR, R.A. and BURKE, C.W. 1976. Triiodothyronine and thyroxine in urine. I. Measurement and application. J. Clin. Endocrinol. Metab. 42:494-503.

SLEBCDZINSKI, A. 1972. Elimination of radioiodine by the sheep parotid gland after (I-131) L-triiodothyronine administration. J. Endocrinol. 52:427-434.

SOFFER, R.L., HECHTMAN, P. and SAVAGE, M. 1973. L-triiodothyronine amino transferase. J.Biol. Chem. 248:1224-1230.

SCKAL, R.R. and ROHLF, F.J. 1969. Biometry. W.H. Freeman and Company, San Francisco.

STANBURY, J.B. and MORRIS, M.I. 1957. The metabolism of 3,3'diiodothyronine in man. J. Clin. Endocrinol. Metab. 17:1324-1331.

VISSER, T.J., LAMBERTS, S.W.J., WILSON, J.H.P., DOCTER, R. and HENNEMAN, G. 1978. Serum thyroid hormone concentration during prolonged reduction of dietary intake. Metab. 27:405-409.

WESTRA, R. and CHRISTOPHERSCN, R.J. 1976. Effects of cold on digestibility, reticulum motility and thyroid hormones in sheep. Can. J. Anim. Sci. 56: 699-708.

WYNN, J. and GIBBS, R. 1964. Thyroxine degradation. J. Biol. Chem. 239:527-529

YOUSEF, M.K. and JOHNSON, H.D. 1968. Effects of heat and feed restriction during growth on thyroxine secretion rate of male rats. Endocrinology 82:353-358.

APPENDIX 1.1

Total I-125 cpm per ml of serum and in the triiodothyronine (T3) fraction of one ml serum following a single intravenous injection of 1,100,000,000 cpm of I-125 T3 (90% pure) into sheep receiving either 1520 or 760 g alfalfa pellets per day. The experiment was conducted in three periods.

Period 1

Feed (g/d)	1520		760	
Sheep No.	8277		8254	
Time (h)	Total	T3	Total	T3
.75	159052	118414	140560	112952
1.00	105832	74534	102545	71634
2.25	60154	45341	61701	50730
3.00	-	-	-	-
4.50	-	-	34083	1043
6.00	40652	2426	30760	11681
8.00	23532	9397	27178	14856
12.00	17947	7565	23169	7864
17.00	14240	5344	22084	8116
24.00[1]	8742	2761	13733	5342
36.00	6259	1640	9564	2105
28.00	4634	580	8058	617
58.50	4325	190	7203	1883
264.00	1165		1604	
288.00	1240		1708	
300.00	598		1035	
356.00	362		802	
600.00	316		627	

1) After this sample there were insufficient counts for counting accuracy of >7%

APPENDIX 1.2

Total I-125 cpm per ml of serum and in the triiodothyronine (T3) fraction of one ml serum following a single intravenous injection of 1,100,000,000 cpm of I-125 T3 (90% pure) into sheep receiving either 1520 or 760 g alfalfa pellets per day. The experiment was conducted in three periods.

Period 2

Feed (g/d)	1520		1520		760		760	
Sheep No.	8254		8281		8277		8288	
Time (h)	Total	T3	Total	T3	Total	T3	Total	T3
.00	17	17	7	7	-2	-2	-7	-7
.33	102512	90058	-17	-3	149692	133343	125808	108274
.66	76412	65190	80148	68505	91396	74291	88462	71973
1.00	60963	50366	60976	51813	78431	63119	78376	63816
1.50	46729	37120	48146	37727	52952	41511	62554	47860
2.00	39436	29411	41209	29965	56631	48026	52576	37493
3.00	33261	22595	34833	22005	44543	25809	39469	23634
5.00	36437	23083	39075	27763	52205	29501	60871	36263
7.00	27116	15966	31870	21625	39239	21784	38528	22405
11.33[1]	20557	11301	21780	15003	29830	14126	24671	12811
17.00	14791	6819	12646	8829	21672	8102	15985	7240
24.00	10006	3756	7908	6500	17475	3978	11075	4415
37.50	5744	831	5449	1299	10816	2596	7810	905
48.00	2924		2090		5075		4536	
60.00	2577		1595		4140		3819	
72.00	2247		1462		4232		3316	
94.00	1603		1052		2843		2827	
118.00	1164		776		3035		3039	
132.00	1073		730		2436		2275	
156.00	879		641		1908		2150	
174.00	628		530		1584		1670	
198.00	724		473		1363		1595	
222.00	556		345		1037		1283	

1) After this sample there were insufficient counts for counting accuracy of >7%

APPENDIX 1.3

Total I-125 cpm per ml of serum and in the triiodothyronine (T3) fraction of one ml serum following a single intravenous injection of 1,100,000,000 cpm of I-125 T3 (90% pure) into sheep receiving either 1520 or 760 g alfalfa pellets per day. The experiment was conducted in three periods.

Period 3

Feed (g/d)	1520		760	
Sheep No.	8240		8290	
Time (h)	Total	T3	Total	T3
.00	-3	-	3	-
.33	180952	163017	245416	217905
.66	114378	101458	187392	174998
1.00	71422	60786	125161	109413
1.50	55547	45192	90774	77178
2.00	36443	29086	63134	52057
3.00	30654	21299	51295	39882
5.00	21063	12267	34602	24079
7.00	15234	9480	25012	15346
11.33	11936	5459	16097	8236
17.00[1]	6763	2196	11566	5110
24.00	6172	1277	7849	2148
35.50	3866	449	6396	507
50.00	2507	-551	5486	149
59.50	2073	-489	5277	-402
73.00	1636	-47	4757	-219
85.00	1205	-432	4102	-351
94.00	1093	-3069	4067	51
118.00	954	-27	3417	-261
142.00	734	-208	2941	-65

1) After this sample there were insufficient counts for counting accuracy of >7%

APPENDIX 2.1

Total I-125 cpm per ml of saliva in the iodide (I) fraction of one ml saliva following a single intravenous injection of 1,100,000,000 cpm of I-125 triiodothyronine (90% pure) into sheep receiving 1520 or 760 grams of alfalfa pellets per day. The experiment was conducted in three periods

Period 1

Feed (g/d)	1520		760	
Sheep No.	8277		8254	
Time (h)	Total	I	Total	I
.00	1	1	1	1
.75	16195	9927	44260	27132
1.00	13107	8588	79424	60130
2.25	28308	18420	73942	68767
3.00	-	-	81856	16371
4.50	27635	22287	-	-
6.00	-	-	-	-
8.00	82836	67415	48612	38694
12.00	82853	69176	33218	29511
17.00	82866	62398	64781	53708
24.00	82911	33479	49711	33914
35.00	65477	61152	28754	8550
50.00	82940	73642	8612	8238
288.00	8068		8120	
480.00	1108		551	

APPENDIX 2.2

Total I-125 cpm per ml of saliva in the iodide (I) fraction of one ml saliva following a single intravenous injection of 1,100,000,000 cpm of I-125 triiodothyronine (90% pure) into sheep receiving 1520 or 760 grams of alfalfa pellets per day. The experiment was conducted in three periods

Period 2

Feed (g/d)	1520		1520		760		760	
Sheep No.	8254		8281		8277		8288	
Time (h)	Total	I	Total	I	Total	I	Total	I
.00	-	-	-	4	-	-	-	-
.50	5838	3032	10539	6863	10123	6713	11875	8267
1.25	9946	6408	10829	14443	14455	10047	-6	-3
2.50	23401	14663	13744	8141	21008	14043	28344	18725
4.00	37671	29011	18363	11691	169884	107513	27249	20691
12.00	44776	34064	26041	17819	54021	42641	47262	36686
18.00	42878	37155	16100	11420	76621	68218	63014	57264
24.00	31316	16841	33796	20151	64315	57563	65890	56237
48.00	35764	29337	23093	19308	35788	29576	59593	54344
60.00	44614	38783	17018	10680	28676	20933	53528	45695
72.00	14246	9336	10944	6963	33295	23792	49761	39914
96.00	8416	3577	6475	3825	22624	15822	51080	
144.00	3266		3519		14975		31548	

APPENDIX 2.3

Total I-125 cpm per ml of saliva in the iodide (I) fraction of one ml saliva following a single intravenous injection of 1,100,000,000 cpm of I-125 triiodothyronine (90% pure) into sheep receiving 1520 or 760 grams of alfalfa pellets per day. The experiment was conducted in three periods

Period 3

Feed (g/d)	1520		760	
Sheep No.	8240		8290	
Time (h)	Total	I	Total	I
.00	-10	-	10	-
.50	11315	8791	12669	9305
1.25	17129	13057	21204	17383
2.50	38542	34015	47075	41025
4.00	31545	24203	70341	54184
7.00	44265	34550	55283	47458
13.00	35427	27384	68065	58268
24.00	29685	22259	74000	58747
48.00	16075	9159	70700	56459
61.00	8054	5912	56711	43476
70.00	5042	3581	54964	44419
96.00	3417	1927	32120	26789
120.00	1988	1455	30493	23254
144.00	5511		6358	

APPENDIX 3.1

Total I-125 cpm, iodide (I) cpm and non-migrating (Origin) cpm per ml of rumen fluid following a single intravenous injection of 1,100,000,000 cpm of I-125 triiodothyronine into sheep receiving either 1520 or 760 g alfalfa pellets per day. The experiment was conducted in three periods

Period I

Feed (g/d)	1520			760		
Sheep No.	8277			8254		
Time (h)	Total	I	Origin	Total	I	Origin
.00	5	5	5	-5	-5	-5
.75	215	53	74	489	172	136
1.00	1444	713	469	914	178	586
2.00	1735	446	964	2271	539	1291
2.25	4560	956	2252	5050	836	3217
3.00	4164	843	2599	6794	1500	4053
4.50	7013	1270	4239	10432	1938	6524
6.00	7081	1507	4121	21522	2703	16053
8.00	12304	5583	5425	30144	12184	15306
12.00	17681	7278	8315	-3	-2	-
17.00	19183	6631	9565	40146	13936	20519
24.00	15881	7879	5465	34249	12378	17054
35.00	9741	3390	4392	20218	7281	9313
50.00	8815			19994		
360.00	816			1390		
450.00	281			677		

APPENDIX 3.2

Total I-125 cpm, iodide (I) cpm and non-migrating (Origin) cpm per ml of rumen fluid following a single intravenous injection of 1,100,000,000 cpm of I-125 triiodothyronine into sheep receiving either 1520 or 760 g alfalfa pellets per day. The experiment was conducted in three periods

Period 2

Feed (g/d)	1520			1520			760			760		
Sheep No.	8254			8281			8277			8288		
Time (h)	Total	I	Origin	Total	I	Origin	Total	I	Origin	Total	I	Origin
.00	4	4	4	-3	-3	-3	5	5	5	-6	-6	-6
.00	-	-	-	-3	-3	-3	7	7	7	-4	-4	-4
1.75	478	806	-41	398	222	42	527	513	-319	502	257	-181
3.50	1981	1348	121	2166	790	311	1340	679	546	2463	722	568
7.00	4461	1680	1259	2177	948	245	5418	1570	1671	5837	1913	1982
12.00	5217	1290	1521	5429	2490	838	6161	1767	2192	9171	3092	3395
19.00	7035	2980	1894	4161	1648	570	9069	1461	4660	9925	1674	5022
24.00	5038	1177	1807	3794	1467	937	7338	1140	2683	11684	2628	5164
36.00	7911	1613	2219	3317	1038	461	8399	1071	3040	11290	1873	4598
49.50	4165	1170	833	3730	1652	231	6634	1561	2334	10493	2186	3349
60.00	2425	737	566	2187	917	195	4653	1393	1887	8476	2264	3539
72.00	2360	667	984	1835	806	301	4772	1587	1700	8203	2277	3848
96.00	1498	681	182	1218	563	239	3887	930	1733	7532	1922	3570
120.00	747	596	51	752	214	210	2662	1554	455	6441	2315	2536
144.00	1063	164	289	860	315	85	3061	1229	635	6027	4405	725
192.00	268	65	16	236	44	6	1636	484	347	4648	1774	1085

APPENDIX 3.3

Total I-125 cpm, iodide (I) cpm and non-migrating (Origin) cpm per ml of rumen fluid following a single intravenous injection of 1,100,000,000 cpm of I-125 triiodothyronine into sheep receiving either 1520 or 760 g alfalfa pellets per day. The experiment was conducted in three periods

Period 3

Feed (g/d)		1520			760	
Sheep No.		8240			8290	
Time (h)	Total	I	Origin	Total	I	Origin
.00	-2	-2	-2	1	1	1
.00	-4	-4	-4	4	4	4
1.75 [1]	2073	1564	210	1306	132	508
3.50	3736	1788	899	4540	2040	1258
7.00	8783	4174	2126	6039	3109	1314
12.00	9964	4970	2243	-41	-23	-8
18.00	-41	-19	-9	-41	-19	-10
24.00	9784	4708	2443	18396	8750	5069
35.00	6875	3441	1642	16664	12124	2191
49.00	5593	2634	1442	13906	6337	4053
59.00 [2]	4985	2274	1133	14282	7989	2126
71.00	3291	1502	726	13541	7969	2126
84.00	2034	1092	398	10732	4195	2840
93.00	1654	835	271	12225	5961	2254
120.00	1142	430	308	11568	6758	959
144.00	602	1198	-37	10744	5813	745
168.00	788			15808		

1,2) The numbers above 1 and below 2 did not have sufficient counts for counting accuracy of > 7%

APPENDIX 4.1

Radioactivity (x 10,000 cpm) of total I-125, estimated extractable iodide and estimated extractable triiodothyronine (T3) excreted in feces of sheep following a single intravenous injection of 1,100,000,000 cpm I-125 T3 (90% pure). The sheep were fed 1520 or 760 g of alfalfa pellets per day, the experiment was conducted in three periods

Period I

Feed (g/d)	1520			760		
Sheep No.	8277			8254		
Time (h)	Total	I	T3	Total	I	T3
8	164189938	44086745	23126327	4792014	1098695	1629389
27	311061541	29422599	234964856	294154443	99641505	118191785
36	37661577	10859243	6560403	32948809	20713893	8231579
45	13717199	2303786	5205501	18705334	17656882	-10460690
53	7890793	-142614	-72090	9001448	12712249	-16863938
60	7427575	3433190	3763304	6565334		
72	8697552			8732481		
80	3462899			4165833		
92	6450681			4946244		
102	3810322			4035723		
113	3140664			5726486		
125	3391009			3289179		
137	4194487			3053286		
147	2514312			2980022		
170	2737674			2808527		

Sufficient counts were not present for counting accurracy of >7%

APPENDIX 4.2

Radioactivity (x 10,000 cpm) of total I-125, estimated extractable iodide and estimated extractable triiodothyorine (T3) excreted in feces of sheep following a single intravenous injection of 1,100,000,000 cpm I-125 T3 (90% pure). The sheep were fed 1520 or 760 g of alfalfa pellets per day, the experiment was conducted in three periods

Period 2

Feed (g/d)		1520			1520			760			760		
Sheep No.		8254			8281			8277			8288		
Time(h)	Total	I	T3	Total	I	T3	Total	I	T3	Total	I	T3
0	0	0	0	0	0	0	0	0	0	0	0	0
9	1255388	358588	568406	21875608	4471384	11161664	2814286	778484	1193486	454302	55298	274175
21	502661308	89601766	163992810	452082152	75233995	197687182	147774958	10423453	71945811	203907465	10420096	99639879
25	81254026	2587k681	25323341	98068649	14042162	47894211	212482785	30405631	72430515	622544632	75715705	241878857
37	108982908	67233648	12274338	123104075	47373040	42735423	112262312	15519377	36664854	102434453	18957249	5431088
50	40620531			41103020			56436416			46556757		
61	11244381			15816822			13651172			15441502		
72	6845318			7518828			9141644			8552758		
94	14017504			11260458			6921483			6607189		
118	16294149			4746537			3803187			4828938		
142	5189535			3971670			2740530			4105867		
166	3266445			1972297			2552283			3456095		
190	3112199			3408635			2112487			4320836		
214	1720962			1111648			1340720			3854571		
238	1308353			1202678			1399978			3100852		
262	1763029			905376			1099061			2608608		
286	2208462			836591			1342376			2630050		
310	1149582			523511			796346			1460961		
334	704796			783938			917972			1275330		
358	679590			463673			683272			1451171		

Sufficient counts were not present for counting accuracy of >7%

APPENDIX 4.3

Radioactivity (x 10,000 cpm) of total I-125, estimated extractable iodide and estimated extractable triiodothyronine (T3) excreted in feces of sheep following a single intravenous injection of 1,100,000,000 cpm I-125 T3 (90% pure). The sheep were fed 1520 or 760 grams of alfalfa pellets per day, the experiment was conducted in three periods

Period 3

Feed (g/d)	1520			760		
Sheep No.	8240			8290		
Time (h)	Total	T3	I	Total	T3	I
7	503534	-42053	-17706	118943	-11518	-9265
12	36776189	2392263	25169672	9990696	2358367	410894
24	14038781	3766915	5470413	91308483	8142012	57841195
36	19822128	8129471	2499027	4472138	935388	1711877
48	2149077	709596	455444	20574380	6584718	4348571
60	912587	131970	165598	4694928	1307186	1091293
72	1242061	303788	258551	3653181	993870	677989
84	786302	68801	309606	2359668	496820	666422
96	299835			1647217		
120	779775			4504858		
144	545320			9136994		
168	802337			3836422		
192	528890			5328805		

Sufficient counts were not present for counting accurracy of > 7%

APPENDIX 5.1

Radioactivity (x 10,000 cpm) of total I-125, extractable unknown (see text) and extractable triiodothyronine (T3) excreted in urine of sheep following a single intravenous injection of 1,100,000,000 cpm of I-125 T3 (90% pure). The sheep were fed 1520 or 760 g of alfalfa pellets per day, the experiment was conducted in three periods

Period 1

Feed (g/d)	1520			760		
Sheep No.	8277			8254		
Time (h)	Total	Unknown	T3	Total	Unknown	T3
0	0	0	0	0	0	0
5	64	39	6	6	3	1
10	2563	1125	492	959	425	47
17	3366	1189	309	1656	501	405
27	1398	775	319	741	231	195
36	400	35	264	542	66	150
45	266	37	174	524	0	192
53	176	52	72	427	52	64
66	123	37	63	400	126	259
73	212			324		
81	126			172		
93	152			345		
103	132			30		
117	95			362		
127	0			320		
139	50			196		
148	54			229		
163	0			0		
211	94			237		
235	0			143		
259	96			313		
283	80			0		
312	105			108		
326	70			175		
360	71			0		
384	64			182		
408	160			202		
432	147			265		
456	0			212		

APPENDIX 5.2

Radioactivity (x 10,000 cpm) of total I-125, extractable unknown (see text) and extractable triiodothyronine (T3) excreted in urine of sheep following a single intravenous injection of 1,100,000,000 cpm of I-125 T3 (90% pure). The sheep were fed 1520 or 760 g of alfalfa pellets per day, the experiment was conducted in three periods

Period 2

Feed (g/d)		1520				1520				760				760		
Sheep No.		8254		Extract-		8181		Extract-		8277		Extract-		8288		Extract
Time (h)	Total	Un-known	T3	able label	Total	Un-known	T3	able label	Total	Un-known	T3	able label	Total	Un-known	T3	able label
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	767	401	175	.84	1066	575	58	.86	2330	1353	171	.85	936	516	277	.86
4	866	548	116	.84	2487	1594	379	.80	3256	2305	438	.89	2950	2008	386	.81
8	1367	929	24	.79	1719	1192	25	.78	2276	1361	439	.89	1845	1348	167	.82
13	1679	760	79[1]	.73	2556	1543	164[1]	.73	2085	1146	112[1]	.78	2305	1677	154	.81
19	1181	606	60	.76	1710	972	161	.74	3328	1887	212	.79	2186	1373	83	.76
24	302	107	16	.70	636	224	53	.70	1602	799	144	.76	724	405	18	.73
36	727	199[1]	52	.66	2001	543[1]	98	.63	1471	674[1]	56	.66	546	166[1]	58	.71
50	418	48	-20	.58	884	151	46	.59	1044	343	17	.65	661	271	-5	.67
60	387	545	-54	.55	610	84	-2	.52	722	272	-278	.57	281	-113	802	.58
72	317	-107	-65	.53	354	-17	-113	.48	558	413	269	.50	225	90	-81	.58
93	924	427	-425	.54	378	47	-81	.51	1749		128	.53	457	-38	-24	.53
117	600			.39	113			.49	1701			.52	460			
141	379				577				1570				1063			
165	321				151				593				864			
189	225				164				681				719			
213	153				100				544				578			
237	122				75				418				333			
261	77				46				468				637			
285	52				0				201				465			
309	78				41				264				246			
331	69				0				132				168			
355	33				16				332				99			

[1] After this sample there were insufficient counts for counting accuracy of >7%

APPENDIX 5.3

Radioactivity (x 10,000 cpm) of total I-125, extractable unknown (see text) and extractable triiodothyronine (T3) excreted in urine of sheep following a single intravenous injection of 1,100,000,000 cpm of I-125 T3 (90% pure). The sheep were fed 1520 or 760 g of alfalfa pellets per day, the experiment was conducted in three periods

Period 3

Feed (g/d)	1520				760			
Sheep No.	8240				8290			
Time (h)	Total	Unknown	T3	Extractable Label	Total	Unknown	T3	Extractable Label
0	0	0			0	0		
0	0	0			0	0		
2	1230	703	204	.88	148	75	34	.85
4	2378	1103	296	.89	12883	5039	1812	.77
7	4522	2279	369	.77	7818	3608	707	.85
12	2183	903	103[1]	.75	9591	4123	410	.97
18	3659	1309	222	.68	7682	3045[1]	407[1]	.67
24	2285	693[1]	145	.68	3231	1150[1]	160	.96
36	2739	426	188	.86	6282	1648	268	.78
50	895	100	45	.60	7134	656	123	.57
59	567	7	25	.59	2405	116	26	.53
71	648	2	20	.40	4768	206	116	.46
96	664	47	29	.55	4270	210	153	.50
118	586	17	40	.58	2187	97	96	.66
144	324	27	22	.65	3374	154	102	.47
168	230	24	30	.53	2447	152	115	.53
192	302	38	85	.54	198	11	7	.37
126	106	6	1	.07	166	6	2	.50
240	135	17	7	.53	260	72	24	.55
264	41	7	6	.47	296	19	10	.59
288	53	8	4	.51	259	18	24	.50
312	36	6	9	.50	305	5	8	.54
316	16	3	4	.49	442	27	30	.52
360	12	3	2	.63	290	14	5	.54
384	28	2	2	.86	187	12	7	.31
408	36	5	2	.56	301	30	16	.57
432	57	5	5	.56	304	41	15	.53
456	19	2	1	.57	177	4	19	.58
480	17				118			

1) After this sample there were insufficient counts for counting accuracy of >7%